Aluminum toxicity in a tropical montane forest ecosystem in southern Ecuador

Inauguraldissertation

der Philosophisch-naturwissenschaftlichen Fakultät

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vorgelegt von

Agnes Rehmus

von Deutschland

Leiter der Arbeit:

Prof. Dr. W. Wilcke

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Bern, 23.02.2015

Der Dekan:
Prof. Dr. G. Colangelo

In Andenken an meinen Vater.

Contents

Danksagung

Rund 10 Jahre war ich Teil der Arbeitsgruppe von Prof. Dr. Wolfgang Wilcke. Angefangen hat alles mit dem Spülen von tausenden von Flaschen als wissenschaftliche Hilfskraft. Mit anderen Worten: Eine Tellerwäscherkarriere!

Ich danke meinem Doktorvater, Prof. Dr. Wolfgang Wilcke, dass er es mir ermöglicht hat in seiner Arbeitsgruppe zu promovieren und für die umfassende Betreuung und Unterstützung. Er hat *immer* Zeit gefunden um in inspirierenden Gesprächen Fragen und Probleme zu klären und meine Manuskripte, Abstracts und Konferenzvorträge zu lesen und zu kommentieren.

Ich bedanke mich bei Dr. Moritz Bigalke, der jederzeit ein hilfreicher Ansprechpartner war und mit seiner Unterstützung bezüglich Laboranalysen und wissenschaftlicher Fragestellungen sehr viel zum Gelingen meiner Dissertation beigetragen hat.

Professor Dr. Martin Kaupenjohann danke ich für die externe Begutachtung meiner Dissertation. Allen Koautoren und anonymen Gutachtern danke ich für ihre kritische Auseinandersetzung mit den Manuskripten, die zu deren Verbesserung beigetragen hat.

Die Durchführung der Arbeit wäre nicht möglich gewesen ohne die Bereitstellung der Versuchsflächen und der Forschungsstation in Ecuador durch Naturaleza y Cultura International (NCI) in Loja, die Forschungsgenehmigung der ecuadorianischen Regierung und die Finanzierung des Projektes (FOR 816, Wi 1601/8-2) durch die Deutsche Forschungsgemeinschaft (DFG).

Bei der Durchführung des Aluminiumtoxizitätsversuchs waren mir die Praktikantinnen Katharina Kitzinger und Karoline Klaus sowie Jose Luis Peña Caivinagua, Orly Mendoza Aguirre und viele weitere Menschen vor Ort in Ecuador eine sehr große Hilfe. Dr. Patrick Hildebrandt und Julio Mora Castillo danke ich für die Bereitstellung der Baumsetzlinge für den hydroponischen Versuch, Dr. Ingeborg Haug und Dr. Jürgen Homeier für ihre Hilfe bei der Bestimmung von Baumarten aus dem ecuadorianischen Bergregenwald und Daniel Kübler für eine umfassende Baumkartierung aus dem Untersuchungsgebiet, mit deren Hilfe das Auffinden der spezifischen Baumarten deutlich erleichtert wurde. Dr. Rainer Rees Mertins und Dr. Bernd Felderer aus der Arbeitsgruppe von Prof. Dr. Rainer Schulin danke ich für ihre Unterstützung bei der Wurzel- und Blattanalyse mit WinRhizo.

Ich bedanke mich bei Dr. Daniela Fischer, Michael Wendler, Hanspeter Dettling, Dr. Adrien Mestrot, Martin Schwarz sowie den wissenschaftlichen Hilfskräften für ihre Unterstützung im Labor, Dr. Sophia Leimer und Martin Schwarz für ihre Hilfe bezüglich statistischer Fragen, R- und LaTeX-Anwendungen, Fabrice Grassein für die Klärung vieler statistischer Fragen und Dr. Benjamin Bandowe für die Bereitstellung seines Laborcomputers für SPSS- und OriginLab-Anwendungen.

Ein ganz herzlicher Dank gilt Alexander Hermann, der sogar Abendstunden für die Erstellung der Abbildung zu Al-Flüssen geopfert hat.

Isabella Geissbühler und Marlis Röthlisberger danke ich für die administrative Unterstützung während des Doktorats.

Ein ganz besonderer Dank gilt allen meinen Arbeitskollegen und Freunden für die tolle Zeit am GIUB.

Ich danke Alexander Pohl für seine grenzenlose Geduld und die vielen Tutorials in diversen Softwareanwendungen und seinen Eltern für die mentale Unterstützung in der Abschlussphase und ihre Hilfe beim Korrekturlesen.

Zu guter Letzt danke ich meiner Familie, besonders meiner Schwester und meiner Mamuś, die mich jederzeit vorbehaltlos unterstützt und motivert haben.

Summary

Aluminum phytotoxicity frequently occurs in acid soils (pH < 5.5) and was therefore discussed to affect ecosystem functioning of tropical montane forests. The susceptibility to Al toxicity depends on the sensitivity of the plant species and the Al speciation in soil solution, which can vary highly depending e.g., on pH, ionic strength, and dissolved organic matter. An acidification of the ecosystem and periodic base metal deposition from Saharan dust may control plant available Al concentrations in the soil solutions of tropical montane rainforests in south Ecuador.

The overall objective of my study was to assess a potential Al phytotoxicity in the tropical montane forests in south Ecuador. For this purpose, I exposed three native Al non-accumulating tree species (*Cedrela odorata* L., *Heliocarpus americanus* L., and *Tabebuia chrysantha* (Jacq.) G. Nicholson) to increased Al concentrations ($0 - 2400\ \mu M$ Al) in a hydroponic experiment, I established dose-response curves to estimate the sensitivity of the tree species to increased Al concentrations, and I investigated the mechanisms behind the observed effects induced by elevated Al concentrations. Furthermore, the response of Al concentrations and the speciation in soil solution to Ca amendment in the study area were determined. In a final step, I assessed all major Al fluxes, drivers of Al concentrations in ecosystem solutions, and indicators of Al toxicity in the tropical montane rainforest in Ecuador in order to test for indications of Al toxicity.

In the hydroponic experiment, a 10 % reduction in aboveground biomass production occurred at 126 to 376 μM Al (EC10 values), probably attributable to decreased Mg concentrations in leaves and reduced potosynthesis. At 300 μM Al, increased root biomass production of *T. chrysantha* was observed. Phosphorus concentrations in roots of *C. odorata* and *T. chrysantha* were significantly highest in the treatment with 300 μM Al and correlated significantly with root biomass, being a likely reason for stimulated root biomass production.

The degree of organic complexation of Al in the organic layer leachate, which is central to plant nutrition because of the high root density, and soil solution from the study area was very high (mean > 99 %). The resulting low free Al concentrations are not likely to affect plant growth, although the concentrations of potentially toxic Al^{3+} increased with soil depth due to higher total Al and lower dissolved organic matter concentrations in soil solutions. The Ca additions caused an increase of Al in the organic layer leachate, probably because Al^{3+} was exchanged against the added Ca^{2+} ions while pH remained constant. The free ion molar ratios of Ca^{2+}:Al^{3+} (mean ratio ca. 400) were far above the threshold (≤ 1) for Al toxicity, because of a much higher degree of organo-complexation of Al than Ca.

High Al fluxes in litterfall ($8.8 - 14.2$ kg ha^{-1} yr^{-1}) indicate a high Al circulation through the ecosystem. The Al concentrations in the organic layer leachate were driven by the acidification of the ecosystem and increased significantly between 1999 and 2008. However, the Ca:Al molar ratios in organic layer leachate and all aboveground ecosystem solutions were above the threshold for Al toxicity. Except for two Al accumulating and one non-accumulating tree species, the Ca:Al molar ratios in tree leaves from the study area were above the Al toxicity threshold of 12.5.

I conclude that toxic effects in the hydroponic experiment occurred at Al concentrations far above those in native organic layer leachate, shoot biomass production was likely inhibited by reduced Mg uptake, impairing photosynthesis, and the stimulation of root growth at low Al concentrations can be possibly attributed to improved P uptake. Dissolved organic matter in soil solutions detoxifies Al in acidic tropical forest soils and a wide distribution of Al accumulating tree species and high Al fluxes in the ecosystem do not necessarily imply a general Al phytotoxicity.

Zusammenfassung

Aluminiumtoxizität tritt in sauren Böden bei pH-Werten < 5.5 auf und wurde als ein möglicher Grund für ein verkümmertes Baumwachstum in tropischen Bergregenwäldern diskutiert. Die Anfälligkeit für Al-Toxizität ist von der Pflanzenart abhängig sowie von der Al-Speziierung, die je nach pH-Wert, Ionenstärke und gelöster organischer Substanz (DOM) in der Bodenlösung stark variieren kann. Eine Versauerung des Ökosystems und periodische Baseneinträge mit Saharastäuben könnten pflanzenverfügbare Al-Konzentrationen in tropischen Bergregenwäldern in Südecuador beeinflussen.

Das Ziel meiner Studie war es, eine potentielle Al-Phytotoxizität in tropischen Bergregenwäldern Südecuadors abzuschätzen. Zu diesem Zweck habe ich einen hydroponischen Versuch mit drei einheimischen, nicht Al-akkumulierenden Baumarten (*Cedrela odorata* L., *Heliocarpus americanus* L., und *Tabebuia chrysantha* (Jacq.) G. Nicholson) unter ansteigenden Al-Konzentrationen durchgeführt, Dosis-Wirkungskurven erstellt um die Al-Sensitivität der Baumarten zu ermitteln und die Mechanismen untersucht, die zu den im hydroponischen Versuch beobachteten Effekten geführt haben. Des Weiteren wurde der Einfluss von Ca-Einträgen in das Ökosystem auf die Al-Konzentrationen und die Speziierung in der Bodenlösung untersucht. Im letzten Schritt habe ich alle bedeutenden Al-Flüsse, Kontrollgrößen der Al-Konzentrationen in Bodenlösungen und Indikatoren der Al-Toxizität im Untersuchungsgebiet ermittelt.

Im hydroponischen Versuch wurde die oberirdische Biomasseproduktion bei Al-Konzentrationen von 126 – 376 μM Al (EC10-Werte) um 10 % reduziert und ist auf eine gestörte Mg-Aufnahme und Verringerung der Photosynthese zurückzuführen. In der Behandlung mit 300 μM Al hat die Wurzelbiomasseproduktion von *T. chrysantha* signifikant zugenommen, ebenso die P-Gehalte in den Wurzeln.

Aluminium in der Bodenlösung aus der organischen Auflage, welche aufgrund der hohen Wurzeldichte eine wichtige Rolle für die Nährstoffaufnahme spielt, war fast vollständig organisch komplexiert (Mittelwert > 99 %). Die Konzentrationen an freiem Al^{3+} stiegen mit der Bodentiefe, da die DOM Konzentration mit der Bodentiefe abnimmt. Jedoch ist eine toxische Wirkung durch die geringen Konzentrationen an freiem Al^{3+} unwahrscheinlich. Calciumeinträge bewirkten einen Anstieg der Al-Konzentration in der Bodenlösung aus der organischen Auflage, vermutlich durch den Austausch von Al^{3+} durch Ca^{2+}-Ionen bei gleichbleibendem pH-Wert. Aufgrund der weitaus stärker ausgeprägten Komplexierung von Al gegenüber Ca waren die molaren Ca^{2+}:Al^{3+}-Ionen Verhältnisse (Mittelwert ca. 400) deutlich über dem Grenzwert (≤ 1) für Al-Toxizität.

Hohe Al-Flüsse im Streufall (8.8 – 14.2 kg ha^{-1} yr^{-1}) deuten auf einen ausgeprägten Al-Kreislauf im Ökosystem. Die Al-Konzentrationen in der Bodenlösung aus der organischen Auflage sind mit der Versauerung des Ökosystems zwischen 1999 und 2008 signifikant angestiegen. Mit Ausnahme von zwei Al-Akkumulatoren und einer nicht-akkumulierenden Spezies, lagen die molaren Ca:Al-Verhältnisse in Blättern von Waldbäumen aus dem Untersuchungsgebiet über dem Al-Toxizitätsgrenzwert von 12.5.

Daraus schließe ich, dass toxische Effekte im hydroponischen Versuch bei Al-Konzentrationen auftraten, die deutlich über den Konzentrationen in der Bodenlösung aus der organischen Auflage liegen. Der Rückgang der oberirdischen Biomasseproduktion mit steigender Al-Konzentration im hydroponischen Versuch wurde durch eine verringerte Mg-Aufnahme und damit reduzierte Photosynthese verursacht. Eine Stimulation der Wurzelbiomasseproduktion bei niedrigen Al-Konzentrationen ist vermutlich einer verbesserten P-Aufnahme zuzuschreiben. Die toxische Wirkung von Al in sauren tropischen Waldböden wird durch Komplexierung mit DOM reduziert. Eine weite Verbreitung von Al-akkumulierenden Pflanzen und hohe Al-Flüsse im Ökosystem implizieren nicht zwangsläufig eine Al-Phytotoxizität.

List of figures

List of tables

List of abbreviations

AAS	atomic absorption spectroscopy
Al electrostat. DOM	Al electrostatically bound to DOM
Al spec. DOM	Al specifically bound to DOM
ALS	aluminum saturation
a.s.l.	above sea level
BD	bulk deposition
BS	base saturation
CB	canopy budget
C. odorata	*Cedrela odorata* L.
CV	coefficient of variation
DD	dry deposition
DMT	Donnan menbrane technique
DOC	dissolved organic carbon
DOM	dissolved organic matter
EC	electrical conductivity
ECEC	effective cation exchange capacity
EC10 – 50	effective concentration at which 10 to 50 % effect occurred
FDR	frequency domain reflectometry
H. americanus	*Heliocarpus americanus* L.
IC	ion chromatography
ICP–MS	inductively coupled plasma mass spectrometry
LF	litterfall
LL	organic layer leachate
MC	microcatchment
n	sample size
NUMEX	nutrition manipulation experiment
PCA	principal component analysis
r	Pearson product-moment correlation coefficient

r_s	Spearman's rank correlation coefficient
SE	standard error
SD	standard deviation
SF	stemflow
SS15, SS30	soil solution at the 0.15 m and 0.30 m soil depth
SW	stream water
T. chrysantha	*Tabebuia chrysantha* (Jacq.) G. Nicholson
TF	throughfall
TN	total N
TOC	total organic carbon
TP	total P
WB	watershed budget

1. Summarizing overview

1.1 Introduction

Aluminum phytotoxicity is known to frequently occur in acid soils at pH values < 5.5 at which plant available Al concentrations in soil solutions increase (Alleoni et al. 2010; Delhaize and Ryan 1995; Kochian et al. 2004; Schaedle et al. 1989). Aluminum toxicity may contribute to forest decline in temperate forests (Cronan 1989; Godbold et al. 1988; Farr et al. 2009) albeit at soil pH values lower than in the tropics, where pH usually ranges between 4 and 5 controlled by the Al oxide buffer. Nevertheless, Al toxicity was suggested to contribute to low biomass production and slow nutrient-cycling rates in tropical montane forests (Bruijnzeel 2001; Bruijnzeel and Veneklaas 1998; Hafkenscheid 2000; Leuschner et al. 2007). However, hypotheses relating the stunted growth of tropical montane forests to Al toxicity were up to the present study never directly tested by dose-effect experiments with tropical forest trees.

When assessing Al toxicity, not the total Al concentrations in soil solutions should be addressed, because Al can occur in various toxic and non-toxic species, depending on pH, dissolved organic matter (DOM) and ionic strength of the solution (Kinraide 1991, 1997). The free Al^{3+} ion, the mononuclear $Al(OH)_2^+$ and $Al(OH)^{2+}$ ions and polynuclear Al species (e.g., $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$ in the literature referred to as Al_{13}) are phytotoxic. Fluoride complexes (AlF^{2+} and AlF_2^+) are considered to be toxic as well, while sulfate complexes and organically complexed Al are supposed to be nontoxic (Alva et al. 1986; Buyukkeskin and Akinci 2011; Cronan and Grigal 1995; Kinraide 1997).

For economic reasons, many studies on Al toxicity have been conducted on crop plants (Kochian et al. 2004; Ryan et al. 2011; Schaedle et al. 1989) and tree species used for afforestations (Kinraide 2003; Schaedle et al. 1989). Only few tropical forest tree species have been tested for their susceptibility to Al toxicity (Cuenca et al. 1990; Watanabe et al. 1998). Cuenca et al. (1990) described adaption mechanisms of tropical trees to Al stress in acidic soils and differences in Al accumulating trees compared to Al non-accumulating trees. Watanabe et al. (1998) investigated the effect of Al on tropical Al accumulating species and found an increase in tree growth at concentrations of 500 μM Al in the growth solution. Stimulation of plant growth induced by toxic metal ions at low concentra-

tions is known as hormesis (Calabrese and Blain 2009; Poschenrieder et al. 2013). Poschenrieder et al. (2013) distinguish three main mechanisms behind growth stimulation, which are I) amelioration by substrate interactions, II) activation of stress specific defenses like lag-time dependent activation of tolerance mechanisms and priming or acclimation by preexposure to low concentrations of the toxic element, and III) activation of general defense mechanisms, for instance against oxidative and biotic stress. A stimulation of plant growth and especially of P but also N and K uptake by application of Al was observed for some Al-tolerant plant species by Osaki et al. (1997). Hajiboland et al. (2013) investigated the mechanisms for Al-induced growth stimulation in an Al-tolerant tea plant (*Camellia sinensis* L. Kuntze) in a hydroponic experiment and attributed the growth stimulation to enhanced photosynthesis rates and a better protection of membranes against peroxidation by the activation of antioxidant defense enzymes. Several studies investigated the P-Al interactions in the context of Al-toxicity resp. Al-tolerance (Jiang et al. 2009; Liao et al. 2006; Watanabe and Osaki 2001; Zheng et al. 2005), suggesting both, alleviation of Al toxicity by precipitation of Al-P compounds in the roots or on the root surface as well as stimulated extrusion of specific organic acids, which chelate the trivalent Al cation.

Negative effects of high Al availability include, for instance, inhibition of water and nutrient uptake and plant growth, at which particularly root biomass production is negatively affected (Delhaize et al. 2012; Kochian 1995; Rout et al. 2001; Schaedle et al. 1989; Thornton et al. 1987). Inhibition of root elongation is suggested to be a result of Al stress to the root meristem (Ryan et al. 1993). Additionally, under limiting conditions such as root damage or nutrient stress, the plants assign carbohydrates to roots rather than to plant shoots (Harris 1992; Leuschner et al. 2007). Furthermore, exudation of chelating compounds to counteract Al toxicity is energy consuming and happens at the cost of shoot growth (Cuenca et al. 1990). Aluminum was reported to reduce concentrations of P and K in Al-sensitive plants (Graham 2001; Osaki et al. 1997; Thornton et al. 1987). Reduced uptake and translocation of Mg was also observed in various studies (Graham 2001; Keltjens 1995; Kinraide 2003; Osaki et al. 1997; Thornton et al. 1987). As the central ion of the chlorophyll molecule, Mg has a crucial role in photosynthesis. Magnesium deficiency leads to depressed plant growth, the translocation

of Mg from mature to young leaves and protein degradation, causing chlorosis in mature leaves (Marschner 2012).

At our study sites in the tropical montane forests in southern Ecuador, a wide distribution of Al-accumulating plant species has been observed (Homeier 2008), which have Al concentrations > 1000 mg kg^{-1} in leaves (Chenery 1948). Predominantly, the Al accumulators appertain to the families Rubiaceae and Melastomataceae (Homeier 2008). Aluminum accumulation is usually interpreted as adaptation to high plant-available Al concentrations (Jansen et al. 2002) and thus Al toxicity. Other common and economically important non-accumulating tree species in the montane rain forests of southern Ecuador are *Cedrela sp* (Meliaceae) and *Tabebuia chrysantha* (Jacq.) G. Nicholson (Bignoniaceae), which are long-lived pioneers with slow growth rates (Homeier 2008). They belong to the mid-successional tree species and are typically present in primary old-growth forests (Mosandl and Günter 2008). A representative of early-successional species is *Heliocarpus americanus* L. (Tiliaceae), a light demanding fast-growing short-lived pioneer tree (Homeier 2008). In the present study, a hydroponic experiment was conducted with *C. odorata*, *H. americanus*, and *T. chrysantha* to test the response of native tree species to elevated Al concentrations.

In the tropical montane rain forest of south Ecuador the crucial solution for plant nutrition is the leachate from the thick organic layers (0.15 – 0.6 m), where 51 to 76 % of the fine root length are located (Soethe et al. 2006). In this organic layer leachate dissolved Al concentrations are comparatively low ($< 60\ \mu M$) and geochemical speciation modeling suggested that Al is almost entirely organically complexed (57 – 100 %, mean 99 %) because of high dissolved organic carbon (DOC) concentrations (Wullaert et al. 2013). While Wullaert et al. (2013) did not investigate mineral soil solutions, Alleoni et al. (2010) and Vieira et al. (2009) reported that in subtropical and tropical agricultural soils 30 – 99 % of the Al in soil solution is organically complexed. The high degree of Al complexation in tropical and subtropical soil solutions suggests that despite low pH values, Al toxicity might nevertheless play a small role. However, the thermodynamic speciation modeling of Al used by Alleoni et al. (2010), Vieira et al. (2009), and Wullaert et al. (2013) is based on a number of uncertain assumptions, particularly with respect to the Al-complexing properties of DOM. Therefore, there is

an additional need to directly measure free Al ion concentrations with the Donnan membrane technique (DMT, Temminghoff et al. 2000) to support modeling results.

Seasonal acid deposition originating from Amazonian forest fires (Boy and Wilcke 2008) and an increasing NH_4^+ deposition with subsequent nitrification already in the forest canopy and nitrate leaching through the Ecuadorian forest ecosystem result in acidification of the organic layer leachate (Wilcke et al. 2013a,b). A continuous and pronounced acidification period of 10 years was observed between two strong La Niña events in 1999/2000 and 2009/2010. During La Niña events, base metal deposition and the pH of the precipitation increase strongly and partly reverse the acidification trend (Boy and Wilcke 2008; Wilcke et al. 2013a). An overall acidification of the ecosystem might lead to increased Al mobilization and an increased threat of Al phytotoxicity. Currently, the pH value in the organic layer leachate of the Ecuadorian montane rain forest ranges between 4.6 and 5.2, below the critical value of 5.5 for Al mobilization.

Aluminium toxicity depends on the presence of competing cations such as Ca and Mg in soil solution, which are at high concentrations known to alleviate Al toxicity (Cronan and Grigal 1995; Kinraide 2003). A commonly used approach to estimate the threat of Al-stress to plants is the Ca:Al molar ratio in plant tissue and soil solution and the base saturation of the soil (Cronan and Grigal 1995). According to Cronan and Grigal (1995), indices for a 50 % risk of adverse impacts on tree growth induced by Al stress are a Ca:Al molar ratio ≤ 1.0, ≤ 0.2, and ≤ 12.5 in soil solution, fine root tissue, and foliar tissue, respectively, and a soil base saturation ≤ 15 % of the effective cation exchange capacity (ECEC). Thus, the episodic Ca and Mg inputs originating from the Sahara into the tropical montane forests on the rim of the Amazon basin observed by Boy et al. (2008) might reduce the threat of Al toxicity. To assess the effect of low-level Ca addition on the ecosystem, in 2008 a Ca amendment experiment with annual application of Ca as pH neutral $CaCl_2$ was started (Wullaert et al. 2013) in the frame of the Nutrient Manipulation Experiment (NUMEX, Wullaert et al. 2010; Homeier et al. 2012).

The objectives of this study were to

i) test the response of biomass production and plant morphology of the native tree species *C. odorata*, *H. americanus*, and *T. chrysantha* to elevated Al concentrations in a hydroponic experiment **(Chapter 2)**

ii) unveil the mechanisms leading to the observations in the hydroponic experiment, i.e. aboveground biomass reduction with increasing Al concentrations and stimulated root biomass production of *T. chrysantha* at low Al concentrations in nutrient solution **(Chapter 3)**

iii) determine Al speciation by DMT measurements and thermodynamic modelling and the response of Al concentrations and speciation to Ca amendment in the organic layers and mineral soil solutions **(Chapter 4)**

iv) assess Al phytotoxicity in the studied ecosystem by determination of the Al fluxes and drivers of Al concentrations **(Chapter 5)**.

1.2 Materials and Methods

1.2.1 The hydroponic experiment

Experimental design The hydroponic growth experiment with tree seedlings was set up in a greenhouse at the research station San Francisco (4° 00‘ S, 79° 05‘ W), located in the Reserva Biológica San Francisco on the eastern slope of the Cordillera Real, southern Ecuador. Tree seedlings selected for the experiment were *C. odorata*, *H. americanus*, and *T. chrysantha*, aged 7, 6, and 3 months, respectively. These tree species are currently tested as native alternative species for afforestation to replace the locally common exotic *Pinus sp.* and *Eucalyptus sp.* (Mosandl and Günter 2008). Tree seedlings were raised in a nursery from seeds collected from the local forest and germinated in a 50 % soil-sand mixture. At the start of the hydroponic experiment, roots were prewashed thoroughly with tap water to remove soil and rinsed with distilled water before placed in nutrient solution. Before addition of Al, tree seedlings were grown for two weeks in nutrient solution **(Chapters 2 and 3)**.

Figure 1.1: Setup of the hydroponic experiment in the greenhouse. Culture solutions were aerated with a compressor via tubes. Aeration of each culture solution was regulated with hose clamps

One tenth Hoagland solution (Hoagland and Arnon 1950) was used as the basis for the experiments because it resembles the nutrient composition of the organic layer leachate of the study area. However, an important dissimilarity to the organic layer leachate is that in our nutrient solution P concentrations were about 10 times higher than in organic layer leachate. This implies a better P supply of the plants grown in hydroponic solution than in the forest soil and possibly a related deterioration of the plant availability of Fe and Zn (Marschner 2012; Poschenrieder et al. 2013). Nine replicate seedlings per species were treated with 0, 300, 600, 1200, and 2400 μM Al, which was added as $AlCl_3$ to the nutrient solution. The pH was adjusted to 4 using NaOH and HCl **(Chapters 2 and 3)**. In addition, to simulate plant growth in natural organic layer leachate, one set of replicates of each tree species was treated with an organic layer leachate, without addition of Al. The organic layer leachate used in the experiment was prepared by irrigating a homogenized fresh sample of the whole organic layer from the local forest with distilled water. Each tree seedling was treated with 0.5 L culture

solution. Pots were placed at random and positions changed weekly, when culture solutions were replaced and sampled (mixed sample of the nine replicates per species and treatment). Nutrient solutions were aerated for 15 minutes per hour to ensure aerobic conditions at all times (Fig. 1.1). Of the 162 seedlings in total, only 4 plants died during the experiment (two seedlings of each of *H. americanus* and *T. chrysantha*, i.e. one seedling of each species in each of the 0 and 2400 μM treatments, respectively) and were removed without replacement. After six weeks, plants were harvested, washed thoroughly with distilled water and leaves, stem, and roots were separated **(Chapters 2 and 3)**.

Leaves were scanned immediately after separation at 300 dpi (24 bit) in colour with a Canon scanner (CanoScan LiDE 100) and dried in a drying oven at 55°C to constant weight. Roots were stored cool until scanning with a root scanner (Epson Expression 10000 XL) at 600 dpi (8 bit) grey scale. The scanner was equipped with additional lighting system in the lid to avoid distortion by shadows. Immediately after scanning, roots were dried at 55°C to constant weight. In addition to the dry weight of roots and shoots (stem and leaves combined), the fresh weight of the total plants before and after the experiment (growth rate) was determined. Analysis of healthy and diseased leaf area, total root length, average root diameter, root volume, root tips, and root forks was carried out with WinRhizo 2009 (Regent Instruments Inc., Canada) **(Chapter 2)**.

Chemical analyses Fifty mg of plant material (roots and leaves) were digested in a closed-vessel microwave system (MLS Ethos, Germany). To ensure dissolution of aluminosilicates a digestion with 1.6 mL 69 % HNO_3 , 0.6 mL 30 % H_2O_2, 0.1 mL 48 % HF, and 1 mL 5 % H_3BO_3 was chosen. Concentrations of P, Fe, Mn, Zn, Cu, Ni, Co, and Al in plant digests and nutrient solution were determined with ICP-MS (7700x Agilent Technologies, Germany). Potassium, Ca, and Mg concentrations in plant digests and nutrient solution were determined with AAS (Zeenit700P Analytik Jena, Germany) **(Chapters 2 and 3)**. Carbon, N, and S concentrations were analyzed with a Vario EL Cube (Elementar Analysensysteme, Germany). If root quantity was not sufficient for both, digestion and CNS analysis, sample digestion was preferred **(Chapter 3)**. Total nitrogen and total organic carbon (TOC) in nutrient solution were analyzed with a Vario TOC Cube

(Elementar Analysensysteme, Germany). NH_4^+-N, NO_3^--N, and PO_4^{3-}-P concentrations in organic layer leachate before and after weekly treatment were analyzed with a Continuous Flow Analyzer (CFA AutoAnalyzer 3 HR , SEAL Analytical, Germany) **(Chapter 2)**.

Statistical analyses and calculations A Principal Component Analysis (PCA) with varimax rotation was applied in order to reduce the number of variables and to extract interrelations among the root and shoot parameters. The PCA was conducted for the three tree species combined and included the variables healthy and diseased leaf area, shoot and root biomass (dry weight), root-to-shoot biomass ratio, total root length, root diameter, root volume, root tips, and root forks. As an important morphological property which is considered indicative of toxicity effects, the root-to-shoot biomass ratio was included in addition to root and shoot biomass (Graham 2001) **(Chapter 2)**.

Differences among treatments were tested using one-way ANOVA and post-hoc tests. When ANOVA residuals were normally distributed (Shapiro-Wilk normality test) and showed homogeneity of variances (Bartlett test of homogeneity of variances), as post-hoc test Fisher's least significant difference (LSD) test with Bonferroni correction was chosen for equal group n (*C. odorata*) and Tukey's honest significant difference (HSD) test for unequal group n (*H. americanus* and *T. chrysantha*, because of the loss of two replicates of each of these species). When normal distribution and homogeneity of variances could not be assumed, the Games-Howell test was used. Differences in concentrations of NH_4^+-N, NO_3^--N, and PO_4^{3-}-P in organic layer leachate before and after weekly treatment were tested with the Mann-Whitney U test. Differences in P concentrations of roots and shoots were tested with Welch's two sample t-test. To test for correlations between selected variables a Pearson Product Moment Correlation was chosen for variables which showed normal distribution. For variables which did not show normal distribution, a nonparametric Spearman's rank correlation (r_s) was conducted. Significance was set at $p < 0.05$ unless otherwise indicated. Statistical analyses were carried out mainly with R 2.13.1 for Windows GUI front-end (R Foundation, Austria). The PCA was performed with the function *principal()*, the ANOVAs with the function *aov()*, the Shapiro-Wilk test with the function *shapiro.test()*,

the Bartlett test with the function *bartlett.test()*, the Fisher's LSD test with the function *LSD.test()*, the Tukey's HSD test with the function *TukeyHSD()* **(Chapter 2)** and *HSD.test()* **(Chapter 3)**, the Mann-Whitney U test with the function *wilcox.test()*, the t-test with the function *t.test()*, and the Pearson and the Spearman's correlation with the function *cor()* and respective modification of the method. For the Games-Howell test SPSS 19 (IBM Corp., United States) was used **(Chapters 2 and 3)**.

Dose-response curves (DRC) were fitted using log(x+1)-transformed Al concentrations in nutrient solutions vs. total biomass (dry weight), healthy leaf area, number of root tips, root diameter, root-to-shoot biomass ratio and diseased leaf area (in % of total leaf area). For the number of root tips a function could only be fitted for *H. americanus*. Fitting failed for root diameter of *T. chrysantha*, for root-to-shoot biomass ratio of *H. americanus*, and for diseased leaf area of *C. odorata*. Effective concentrations (EC) were calculated for 10, 20, and 50 % effect (reduction or enhancement) compared to control. The treatment with organic layer leachate was considered as a supplemental experiment and was excluded from ANOVA, PCA, and DRC fitting, because strong differences in chemical composition of the solutions complicated a comparison. Dose-response curves were fitted with Origin 8.5 for Windows (OriginLab Corporation, USA) **(Chapter 2)**.

1.2.2 Aluminum in the south Ecuadorian montane forest ecosystem

Study site and sampling procedures The two study sites are an approx. 9 ha-large microcatchment (MC 2) between 1900 and 2010 m a.s.l., 30 – 50° steep, and the control and Ca addition plots of the close-by NUMEX at an altitude between 2020 and 2120 m a.s.l., 25 – 84° steep. Both study sites are located on the eastern slope of the eastern Andean cordillera (Cordillera Real) in southern Ecuador between the cities of Loja and Zamora (4°00' S and 79°05' W). The underlying bedrock is interbedding of palaeozoic phyllites, quartzites, and metasandstones, from which mainly Stagnic Cambisols (Hyperdystric, Chromic) at the NUMEX site and Dystric Cambisols (IUSS Working Group WRB 2007) at MC 2 developed. At higher positions of MC 2, Folic Histosols can be found.

However, the Histosols did not originate from high groundwater levels but were formed by thick and largely well aerated organic layers because of high litterfall rates (ca 10 kg ha^{-1} yr^{-1}) and slow organic matter turnover (Wilcke et al. 2002). In the period January 1998 to December 2010, mean annual precipitation was 1950 mm. June tended to be the wettest month, with 240 mm of precipitation on average, in contrast to 116 mm in November, the driest month. The mean temperature at 1950 m above sea level was 16.0°C. The coldest month was July, with a mean temperature of 14.5°C; the warmest was November, with a mean temperature of 16.8°C (personal communication Thorsten Peters, Paul Emck, Rütger Rollenbeck, Jörg Bendix, and Michael Richter). The vegetation is classified as lower montane forest (Bruijnzeel and Hamilton 2000) **(Chapters 4 and 5)**.

Each of the untreated control and the Ca addition plots of NUMEX was fourfold replicated. The unfertilized control plots were located upslope to avoid nutrient leaching from fertilized to control plots. The location of each plot was selected in a way that the vegetation was representative of the area and similar on all plots. Each plot was 400 m^2 (20 m x 20 m) large and the distance between the plots was at least 10 m. The Ca-addition plots received 10 kg Ca ha^{-1} yr^{-1}, split in two applications per year. This is equivalent to ca. 2.5 times the mean annual Ca deposition with rainfall of 4.0 kg ha^{-1} yr^{-1} between 1999 and 2009. Calcium additions started in January 2008, followed by further applications in August 2008, February 2009, July 2009, and February 2010. Calcium was applied manually in solid phase as a fine-grained $CaCl_2 \cdot 2H_2O$ salt in pro analysi quality **(Chapter 4)**.

Three zero-tension lysimeters per plot were used to collect leachate from the organic layer (organic layer leachate (LL) below Oi, Oe, and Oa horizons). The lysimeters were made of plastic boxes with a 0.15 m x 0.15 m collection area and covered with a polyethylene net with a mesh width of 0.5 mm. The lysimeters were introduced from the wall of soil pit to minimize disturbance of the organic layer. The lysimeters were pushed ca. 0.50 m into the soil so that they were entirely covered by the organic layer. Soil solution at the 0.15 m and 0.30 m (SS15 and SS30, respectively) mineral soil depths was collected using suction lysimeters (ceramic suction cups with 1 μm pore size). Soil solution samples were

collected fortnightly between September 2009 and October 2010. To guarantee Al saturation of the ceramic cups to prevent Al sorption to the ceramic material during the sampling period, soil solution was extracted from August 2007 on allowing for equilibration of the cups during two years **(Chapter 4)**.

The ecosystem flux measurements at MC 2 are concentrated along three ca. 20 m-long transects considered representative for the whole catchment, covering about 10 m in elevational difference at 1900 – 1910, 1950 – 1960, and 2000 – 2010 m a.s.l. (MC 2.1, MC 2.2, and MC 2.3). Incident precipitation was collected with Hellmann-type collectors, first at one clearing in the study area, in May 2000 extended to 3 measurement stations, each equipped with five samplers. Throughfall precipitation was first collected with five, since Mai 2000 with eight, and since Mai 2003 with twenty Hellmann-type collectors at each of the three measurement transects (MC 2.1 – 2.3). Stemflow was collected with a polyurethane foam collar on five representative large trees in MC 2.1 and surface flow was measured with a V-shaped weir at the outlet of the stream drainage MC 2. The weir collected all water drained from the whole catchment (Fleischbein et al. 2006). There were no indications that the catchment was not dense or received additional water from outside (Goller et al. 2005; Fleischbein et al. 2006). Furthermore, surface flow outside the stream never occurred (own observation). Water levels at the weir were recorded hourly with a pressure gauge and additionally measured manually after the stream water samples were collected. Suspended matter in river export was volume-sampled twice weekly between 2001 and 2003 at the V-notch weir (250 mL runoff water per sampling). Suspended matter was concentrated by accumulative filtering of the stored solutions at 0.45 μm pore size (3 months runoff per single filter). Further instrumentation (to collect litter leachate, soil solutions, and litterfall) was placed along the measurement transects (MC 2.1 – 2.3). Because the whole catchment was below the condensation level at 2200 m a.s.l. (Bendix et al. 2006), there was no significant difference in throughfall and litterfall among the three transects. Soil solution was collected with three zero-tension lysimeters below the organic layer (LL) and with three suction cups at 0.15 m and 0.30 m depths (SS15 and SS30, respectively) in the mineral soil of each of the three transects, as described above for NUMEX. To avoid adsorption/desorption effects in soil solution, a vacuum was applied on the suction cups after installation allowing

for equilibration of the cups and the soil solution of the first month was discarded. According to Guggenberger and Zech (1992), equilibrated ceramic suction cups do not show significant interactions with the soil solution **(Chapter 5)**.

Weekly sample replicates from MC 2 were bulked to a composite sample per measurement site prior to chemical characterization. Soil water content measurements with FDR (frequency domain reflectometry) probes at transect MC 2.1 at the 0.1, 0.2, 0.3, and 0.4 m depths were used for calculation of soil water fluxes of the three sampling sites (MC 2.1 – MC 2.3, Fleischbein et al. 2006). Each of the three measurement transects was equipped with three litter collectors with the dimension 0.3 m x 0.3 m and 0.5 mm mesh size. Samples were collected weekly and dried to constant mass at 40°C in an oven. The sample mass was recorded for each collector separately; the samples were then bulked to a monthly sample per microcatchment which was chemically characterized. Subject of the present investigation were in general the monthly means from April 1998 to March 2003. In the case of soil solutions, the investigated time interval was May 2000, when soil solutions were sampled for the first time, to April 2003. The organic layer leachate was analyzed from April 1998 to December 2007 and September 2009 to April 2010, with smaller gaps (6 %) in between due to missing samples. Samples of fresh tree leaves representing the most abundant tree species from the study area were collected in two sampling campaigns between October 2005 and February 2006 and in October 2011 from 21 and 9 individual trees, respectively (17 tree species in total, Tab. 5.1). The base and Al saturation of the cation exchange capacities of the mineral soils was determined in the A horizons of 23 soils. Three of the soil samples (collected at our measurement transects) were composited from three separate samplings with soil pits at the lower and upper ends and in the middle of the transects MC 2.1 to MC 2.3, 10 soils were located near the stream draining the watershed between 1880 and 2100 m a.s.l. (valley bottom) and further 10 soils were located near the ridge between 1890 and 2110 m a.s.l. We considered the selected data set of soils as representative for the whole catchment **(Chapter 5)**.

Chemical analyses In Numex LL and mineral soil solutions, electric conductivity (ProfiLine Cond 3110, WTW GmbH, Weilheim, Germany) and then pH

(Sentix HWS, WTW GmbH, Weilheim, Germany) was measured immediately after sampling in an unfiltered aliquot of each sample within < 24 h. Another aliquot was filtered (ashless folded paper filters with pore size 4 – 7 μm, Type 389; Munktell & Filtrak GmbH, Bärenstein, Germany) and frozen until further analyses. Concentrations of Ca, K, Mg, and Na were determined with flame atomic absorption spectrometry (Varian AA240FS, Thermo Fisher, Darmstadt, Germany or ZEEnit 700P, Jena Analytik, Jena, Germany). Samples were furthermore analyzed for concentrations of total dissolved Cl^-, NH_4^+, NO_3^-, and PO_4^{3-} using continuous flow analysis (CFA, AutoAnalyzer 3, Seal Analytical GmbH, Norderstedt, Germany). Sulfate was determined by ion chromatography (Dionex ICS-900, Thermo Scientific, Waltham, MA, USA) after filtration (0.45 μm pore size). Fluoride concentrations were determined with an ion-sensitive electrode (WTW Inolab pH/Ion 735 with a WTW F800 electrode, WTW, Weilheim, Germany) after addition of TISAB III (Fluka Analytical) by standard addition. Dissolved organic carbon concentrations in LL were measured with a high temperature TOC analyzer (varioTOC cube, Elementar Analysensysteme, Hanau, Germany) **(Chapter 4)**.

In ecosystem solutions of MC 2, the concentrations of Cl^- were determined with a Cl^--specific ion electrode (Orion 9617 BN, Thermo Fisher Scientific, Waltham, USA) immediately after collection in Ecuador during the first 3 years. In the fourth and fifth year, Cl^- was analyzed with a segmented Continuous Flow Analyzer (CFA, San plus, Skalar, Breda, Netherlands). Total organic carbon (TOC) concentrations were analyzed with a TOC-5050 (Shimadzu, Düsseldorf, Germany). The Ca, Mg, K, and Na concentrations were determined with atomic absorption spectroscopy (AAS, SpectraAA400, Varian, Darmstadt, Germany and Zeenit700P, Analytik Jena, Jena, Germany), the Al concentrations with inductively coupled plasma mass spectrometry (ICP-MS, VG PlasmaQuad PG2 Turbo Plus, Thermo Fisher Scientific, Waltham, USA and 7700x Agilent Technologies, Frankfurt am Main, Germany). Litter- and leaf samples were digested with 65 % HNO_3 (leaf samples of 2005/2006) or 69 % HNO_3/ 30 % H_2O_2/ 48 % HF/ 5 % H_3BO_3 (leaf samples of 2011) in a closed vessel microwave system (MARS Xpress, Kamp-Lintfort, Germany and MLS Ethos, Leutkirch, Germany, respectively) after drought and homogenization with a ball mill. Calcium, Mg and

Al in leaf digests from the first sampling were analyzed with AAS (AA240FS, Varian, Darmstadt, Germany). In leaf digests from the second sampling Ca and Mg were analyzed with AAS (Zeenit 700P, Analytik Jena, Jena, Germany) and Al with ICP-MS 7700X (Agilent Technologies, Santa Clara, CA). Concentrates of particulate matter from the stream water were digested with 65 % HNO_3/ 48 % HF and measured by AAS for metal content. The base saturation in A horizons of the soils was determined by extraction with 1 M NH_4NO_3 (soil:solution ratio 1:25). Calcium, K, Mg, Na, Al, Fe, and Mn were determined with AAS (SpectrAA400, Varian, Darmstadt, Germany). The pH values were determined in 0.01 mol L^{-1} $CaCl_2$ (soil:solution ratio 1:2.5) with a standard pH electrode (Orion U402-S7, Thermo Fisher Scientific, Waltham, USA) **(Chapter 5)**.

Donnan membrane technique To validate the results of the chemical speciation modeling, we determined the free Al concentrations in soil solutions using the Donnan membrane technique (DMT; Sigg et al. 2006; Temminghoff et al. 2000; Weng et al. 2004). Prior to application, all material used for the DMT was carefully cleaned in an acid bath and rinsed three times with deionized water ($>$ 18.2 MΩ). Afterwards, 600 – 2000 mL of the sample solution (donor solution) were filled in a plastic beaker and placed on a magnetic stirrer. A Donnan membrane field cell which consists of an acryl frame with two negatively charged membranes (BDH Prolabo, No. 55165 2U) which enclose an acceptor solution (Weng et al. 2004) was sunk into the sample in a way that membranes on both sides were accessible for the donor solution. The beaker was sealed with parafilm and covered with a black plastic bucket to protect it from light. The ionic strength of the acceptor solution was adjusted according to the mean ionic strength in the sample solution using $CaCl_2$ (Trace select, Sigma Aldrich, Buchs, Switzerland). After four days of equilibration (Weng et al. 2004), the cells were recovered and the acceptor solution was sampled and directly acidified. The donor solution was characterized in the same way as the soil solution samples. In the acceptor solutions, Al and Na concentrations were determined. Aluminum concentrations were corrected for ionic imbalances between donor and acceptor solutions using Na concentrations (Temminghoff et al. 2000). Because only positively charged ions diffuse through the negatively charged membranes, the corrected Al con-

centrations in the acceptor solutions indicate concentrations of 'free' cationic Al species (Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^+$, AlF^{2+} etc.), which are considered as phytotoxic. Most nontoxic, neutral and negatively charged species e.g., Al complexed by DOM, are not transferred to the acceptor solution (Temminghoff et al. 2000; Weng et al. 2002). The DMT experiment was realized shortly after sampling directly in our field laboratory in Ecuador. Overall, we conducted 12 DMT measurements (at least in triplicate per solution type, LL, n = 4; SS15, n = 3; SS30, n = 5) **(Chapter 4)**.

Speciation modeling and statistics The speciation of Al in LL was modeled with measured data for solution composition (Al, Ca, Cl^-, DOC, EC, F^-, K, Mg, Na, NH_4^+, NO_3^-, pH, PO_4^{3-}, SO_4^{2-}) using the geochemical code Visual MINTEQ (VMINTEQ, Version 3.0 beta, J.P. Gustafsson). Within VMINTEQ, the NICA-Donnan model was used to assess complexation of Al with humic substances. Details about the NICA-Donnan model are given in Kinniburgh et al. (1996). NICA-Donnan properties of metal complexation by organic acids were taken from the literature (Milne et al. 2003). An active DOM/DOC ratio of 2 was assumed and the dissolved organic acids were adjusted to be 100 % fulvic acids. Toxic polynuclear Al species (e.g., Al_{13}, Kinraide 1991) are not considered in the model. However, Al_{13} was not supposed to exist in solution in the forest soil because no strong changes in Al concentrations or pH occurred and total dissolved Al concentrations were generally low. Aluminum speciation was modeled for 308 samples of LL, SS15, and SS30. The F^- concentrations were determined for a subset of 176 samples from all three solution types and were in all cases below limit of quantification (0.81 μM) and in 89 % of the cases even below the limit of detection (0.26 μM). Thus, for the calculation we set F^- concentrations of all samples to half of the detection limit (0.13 μM). Sulfate concentrations were only available for 31 samples. For all samples without measured SO_4^{2-} concentrations, values in the model were set to 0.61 mg L^{-1} (i.e. the mean sulfate concentration of the measured samples). Ionic strength was estimated from electrical conductivity (Tab. 4.1) according to Griffin and Jurinak (1973). A Bonferroni-corrected Kruskal-Wallis test was used to test for significant differences between Ca amended and control plots and among LL and soil

solutions from the different soil depths (Tab. 4.3 and 4.4). Corresponding leached ions in solutions of Ca amended plots were tested with Pearson Product Moment Correlations **(Chapter 4)**.

The Al fluxes were calculated for 5 consecutive hydrological years from April 1998 to March 2003 (in the case of soil solutions 3 years from May 2000 to April 2003). Data gaps in surface flow caused by logger breakdowns were replaced by modeled values from the hydrological modelling program TOPMODEL (Beven et al. 1995) as described in Fleischbein et al. (2006). Data gaps of soil water fluxes (because of lacking soil water contents) were substituted with the help of a regression model of weekly soil water fluxes on weekly throughfall volumes ($R^2 = 0.85$). The Al canopy budget and total Al deposition was estimated with the canopy budget model of Ulrich (1983) using Cl^- as inert tracer (see Boy and Wilcke (2008) for a detailed description). Annual Al and Cl fluxes were calculated based on monthly mean concentrations averaged from usually 4 – 5 weekly measurements. If one monthly concentration per year was missing, which was the case for Al in litterfall at all three measurement transects in November 1999, in August 2002 at MC 2.3 in SS15 and SS30, and in February 2003 at MC 2.2 in SS15 and SS30, the value was substituted by the mean of the previous and the consecutive monthly concentration. If more than one monthly concentration per year was missing, which was the case for Cl in rainfall, throughfall at all three measurement transects, and stemflow in the years 2000 and 2001, the annual fluxes were calculated with volume-weighted means. The dissolved Al catchment budget was calculated by subtracting the annual total deposition (i.e. bulk and fine particulate dry deposition) from aluminum fluxes in stream water **(Chapter 5)**.

We performed multiple regressions among monthly Al concentrations and Ca:Al molar ratios, respectively, with pH values and TOC concentrations in the soil solutions, and additionally with soil moisture in case of the organic layer leachate. The soil moisture was considered because it influences the Al concentrations via concentration/dilution effects. Regression residuals were tested for normal distribution with the Shapiro-Wilk test and for homogeneity of variances with the Breusch-Pagan test. Only variables with a significant influence on Al concentrations were used in the resulting linear model **(Chapter 5)**. We square-

root transformed Al concentrations in organic layer leachate and the soil solution at 0.30 m soil depth and Ca:Al molar ratios in soil solution at the 0.15 m and 0.30 m depth, because of a data skewness > 0.5 (Webster 2001). The Ca:Al molar ratios in organic layer leachate and TOC concentrations in the soil solution at 0.30 m depth were log-transformed because of a data skewness > 1. Furthermore, we eliminated outliers (values greater than 1.5 times the interquartile range) in Ca:Al molar ratios of organic layer leachate (n = 4) and Al concentrations (n = 1) and Ca:Al molar ratios (n = 1) in the soil solution at 0.30 m depth. To test if Al concentrations in organic layer leachate were following an increasing or decreasing temporal trend from 1998 to 2010, a seasonal Mann-Kendall test which accounts for seasonal autocorrelation of the data was conducted. Trends in monthly Al concentrations were tested for the whole investigation period and additionally starting from the La Niña event in 1999 until the end of the continuous acidification period in December 2007. The base saturation and saturation of exchangeable Al was calculated as the proportion of charge equivalent of extractable Ca + K + Mg + Na and Al of the ECEC **(Chapter 5)**.

Statistical analyses were carried out with R 3.0.2 (R Core Team 2013). For the Kruskal-Wallis test and the Pearson correlation the functions *kruskal()* and *cor()* were used **(Chapter 4)**. To test for temporal trends the function *SeasonalMannKendall()*, for multiple regressions the function *lm()*, for the Shapiro-Wilk test the function *shapiro.test()*, and for the Breusch-Pagan test the function *ncvTest()* was used **(Chapter 5)**.

1.3 Results and discussion

1.3.1 Aluminum toxicity to tropical montane forest tree seedlings in southern Ecuador: response of biomass and plant morphology to elevated Al concentrations (Chapter 2)

Increasing Al concentrations in nutrient solutions caused a decrease in shoot biomass and healthy leaf area by 44 % to 83 % at 2400 μM Al. The root biomass

in contrast did not respond (*C. odorata*), declined by 51 % (*H. americanus*), or was stimulated at low Al concentrations of 300 μM (*T. chrysantha*). The EC10 (i.e. reduction by 10 %) values of Al for total biomass were 315 μM (*C. odorata*), 219 μM (*H. americanus*), and 368 μM (*T. chrysantha*). *Heliocarpus americanus*, a fast growing pioneer tree species, was most sensitive to Al toxicity. Plants grew generally worse and showed more damages when grown in organic layer leachate than in treatments with Hoagland nutrient solution, irrespective of the Al concentration. Plant-available N and P concentrations in the organic layer leachate tended to decrease during the treatment, indicating that either N and/or P uptake is complicated or that complexation of essential micronutrients by organic compounds could pose problems influencing plant growth more than dissolved Al. I conclude that Al toxicity occurred at Al concentrations far above those in native organic layer leachate.

1.3.2 Aluminum toxicity to tropical montane forest tree seedlings in southern Ecuador: response of nutrient status to elevated Al concentrations (Chapter 3)

Concentrations of macronutrients in leaves either increased (N, K) with increasing Al concentrations in nutrient solution or decreased (P, Ca, Mg, S). The Mg concentrations in leaves and roots treated with 600 μM Al and more were close to and even below the threshold for nutrient deficiency (1.5 mg g^{-1}, Marschner 2012). Thus, Mg deficiency might cause reduced photosynthesis and inhibited biomass production. Phosphorus concentrations in roots of *C. odorata* and *T. chrysantha* were significantly highest in the treatment with 300 μM Al and correlated significantly with root biomass. This effect would be in line with hormesis because of substrate amelioration according to Poschenrieder et al. (2013). Improved P supply by elevated Al concentrations below toxicity thresholds might even be more important in the P-poorer organic layer leachates. Shoot biomass production was likely inhibited by reduced Mg uptake, impairing photosynthesis. The stimulation of root growth at low Al concentrations can be possibly attributed to improved P uptake.

1.3.3 Response of Al concentrations and speciation in soil solution to Ca amendment in a tropical montane forest in southern Ecuador (Chapter 4)

Modeled (here and in the following mean±SE: LL 0.56±0.21 %, SS15 0.53±0.25 %, SS30 0.87±0.32 % of total Al are cationic Al species) and DMT-measured free Al concentrations (LL 0.68±0.41 %, SS15 0.52±0.20 %, SS30 0.93±0.26 % of total Al are cationic Al species) showed good agreement. The degree of organic complexation of Al was very high (99.58±0.09 %). The resulting very low free Al concentrations are not likely to affect plant growth. The concentrations of potentially toxic Al^{3+} increased with soil depth (0.00±0.00 μM, 0.01±0.01 μM, and 0.25±0.06 μM in LL, SS15, and SS30 respectively), because of higher total Al and lower DOC concentrations in soil solutions. The Ca additions caused an increase of Al in LL, probably because Al^{3+} was exchanged against the added Ca^{2+} ions while pH remained constant. The total Ca:total Al molar ratios were close to 1 and sometimes < 1 which is usually interpreted as indication of the risk of Al toxicity (Cronan and Grigal 1995). However, free ion molar ratios of Ca^{2+}:Al^{3+} were wide (374±69), because of a much higher degree of organo-complexation of Al than Ca. Concluding, dissolved organic matter concentrations detoxify Al in acidic tropical forest soils.

1.3.4 Aluminum cycling in a tropical montane forest ecosystem in southern Ecuador (Chapter 5)

High Al fluxes in litterfall (8.77±1.3 – 14.2±1.9 kg ha^{-1} yr^{-1}, mean±SE) indicate a high Al circulation through the ecosystem. The Al concentrations in the organic layer leachate were driven by the acidification of the ecosystem and increased significantly between 1999 and 2008. However, the Ca:Al molar ratios in organic layer leachate and all aboveground ecosystem solutions were above 1, the threshold for Al toxicity (Cronan and Grigal 1995). Except for two Al accumulating and one non-accumulating tree species, the Ca:Al molar ratios in tree leaves were above the Al toxicity threshold of 12.5 (Cronan and Grigal 1995). The fraction of exchangeable and potentially plant available Al in mineral soils was high,

being a likely reason for a low root length density in the mineral soil. The results demonstrate that a wide distribution of Al-accumulating tree species and high Al fluxes in the ecosystem do not necessarily imply a general Al phytotoxicity.

1.3.5 Can we expect Al toxicity in the tropical montane forests of south Ecuador?

Until the present study, little effort was made to test for Al phytotoxicity in tropical montane forests. Nevertheless, Al toxicity was discussed as a possible explanation for stunted tree growth in tropical montane forests (Bruijnzeel 2001; Bruijnzeel and Veneklaas 1998; Hafkenscheid 2000; Leuschner et al. 2007). The present study sheds light on the Al toxicity question. Because of the highest root density in the organic layers containing 51 to 76 % of the fine root length (Soethe et al. 2006), primarily the Al concentrations in the organic layer leachate should be addressed in order to assess Al toxicity. However, the remaining roots are distributed in the mineral soil and the Al concentrations in mineral soil solutions should not be disregarded.

The results of the hydroponic experiment with seedlings of the three local tree species *C. odorata*, *H. americanus*, and *T. chrysantha* illustrate that the sensitivity to plant available Al concentrations is tree-species specific and general thresholds can be misleading. For instance, a 10 % reduction of aboveground biomass production in the hydroponic experiment occurred at 126, 238, and 376 μM (*H. americanus*, *C. odorata*, and *T. chrysantha*, respectively) which is a difference of the factor 3 between the most and the least sensitive of the studied tree species (Fig. 1.2 a). However, the mean total Al concentrations in the organic layer leachate from the study site in south Ecuador were 16.2 μM (range $<$ 1 to 58 μM Al, MC 2) and 10.8 μM (range 1.1 to 27.7 μM Al, NUMEX). Consequently, even at peak Al concentrations the lowest EC10 value determined for the native tree species was not reached. At low Al concentrations (300 μM), root biomass production of *T. chrysantha* significantly increased in the hydroponic experiment, illustrating a beneficial effect of Al rather than toxicity. The root biomass production of the other two tree species was not different from the control, demonstrating no negative response to elevated Al concentra-

tions. The coexistence of Al-accumulating tree species with non-accumulators of which the tested tree species showed impaired growth only at Al concentrations far higher than plant available Al concentrations in soil solutions implies that the plants developed different strategies to cope with dissolved Al. For instance, in the hydroponic experiment, TOC concentrations in nutrient solution increased with increasing Al concentrations, indicating exudation of chelating organic acids as a defense mechanism (Brunner and Sperisen 2013). As dissolved Al might occur in various complexes, not the total dissolved Al concentrations but the Al species should be addressed when assessing Al phytotoxicity. The measurement of free Al concentrations with DMT confirms the applicability of Al speciation modelling to soil solutions. The modelling revealed increasing free Al^{3+} concentrations with increasing soil depth from below detection limit up to $< 4\ \mu M$ Al (NUMEX). The high TOC concentrations in organic layer leachate (40 mg L^{-1} in MC 2 and 55 mg L^{-1} in NUMEX) cause an almost complete complexation of Al as organo-Al compounds (mean 99 %) (Fig. 1.2 a). The reason for increasing free Al^{3+} concentrations in mineral soil solutions with increasing soil depth are the decreasing dissolved organic matter concentrations, while pH even increases with increasing soil depth.

Base cations can have ameliorative effects on Al toxicity due to the Ca/Mg-Al antagonism (Cronan and Grigal 1995; Kinraide 2003). In the hydroponic experiment, Al concentrations of 300 μM had to be exceeded to induce significant negative effects on biomass and morphology. In the 300 μM Al treatment, the Ca:Al molar ratios were 1.04, which resembles the suggsted threshold for a 50 % risk of adverse impacts on tree growth induced by Al stress (< 1, Cronan and Grigal 1995). In the 600 μM Al treatment, the Ca:Al molar ratio in nutrient solution was 0.6, approaching the 75 % risk of adverse impacts on tree growth. The mean Ca:Al molar ratios in organic layer leachate were below (NUMEX) the suggested threshold for a 50 % risk of Al toxicity or 10 times higher (MC 2). However, the mean free ion ratios of Ca^{2+}:Al^{3+} in organic layer leachate and mineral soil solutions were 400 times higher than the toxicity threshold (NUMEX, Fig. 1.2 b). Calcium addition to the forest soils in the nutrient manipulation experiment did not increase but decreased Ca:Al molar ratios in organic layer leachate while total Al concentrations increased. The increase of dissolved Al concentrations is

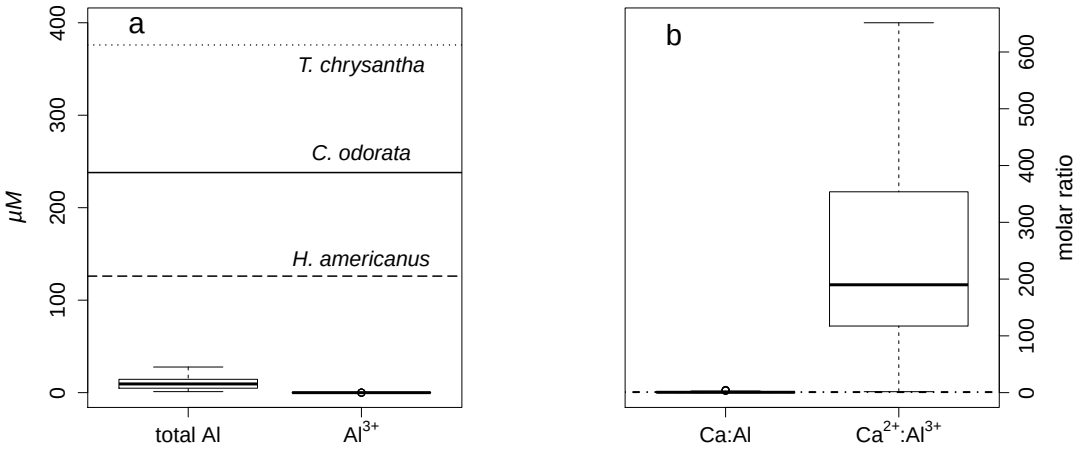

Figure 1.2: Total Al and free Al^{3+} concentrations (μM) in the organic layer leachate in the control plots from the NUMEX study site and EC10 values of *C. odorata*, *H. americanus*, and *T. chrysantha* established in the hydroponic experiment (a) and total Ca : total Al molar ratios and free ion ratios of Ca^{2+}:Al^{3+} in the control plots from the NUMEX study site and the Ca:Al molar ratio (1.0) in the treatment with 300 μM Al (dot-dashed line) in the hydroponic experiment (b), which resembles the suggested threshold of Ca:Al molar ratios for a 50 % risk of adverse impacts on tree growth induced by Al stress (Cronan and Grigal 1995). Free ion ratios of Ca^{2+}:Al^{3+} greater than 700 (values greater than 1.5 times the interquartile range, 18 %) were excluded for illustration purposes

most likely attributable to displacement of Al by Ca from soil exchange sites. Yet, the Ca addition had no effect on the Al speciation.

In the hydroponic experiment, the Mg concentrations in leaves decreased with increasing Al concentrations in nutrient solution (Fig. 1.3 a). Thus, the overall reduction of total biomass production by increased Al concentrations was interpreted as a result of depressed photosynthesis due to decreased chlorophyll which consists of Mg as the central ion of the molecule (Marschner 2012). The Mg concentrations in leaves from the study site were in up to 8 cases below the threshold suggested for optimal plant growth of crop plants (Amberger 1996: 2 – 50 mg g^{-1}; Marschner 2012: 1.5 – 3.5 mg g−1) (Fig. 1.3 a). However, the low Mg concentrations in leaves might not be a result of the Mg-Al antagonism but the Mg-poor bedrock. With few exceptions e.g., in Al accumulators, the Ca:Al molar ratios in tree leaves from the study area and Al treatments in the hydroponic experiment were distinctly higher than the threshold (12.5) for a 50 % risk of toxic affects induced by Al (Cronan and Grigal 1995, Fig. 1.3 b).

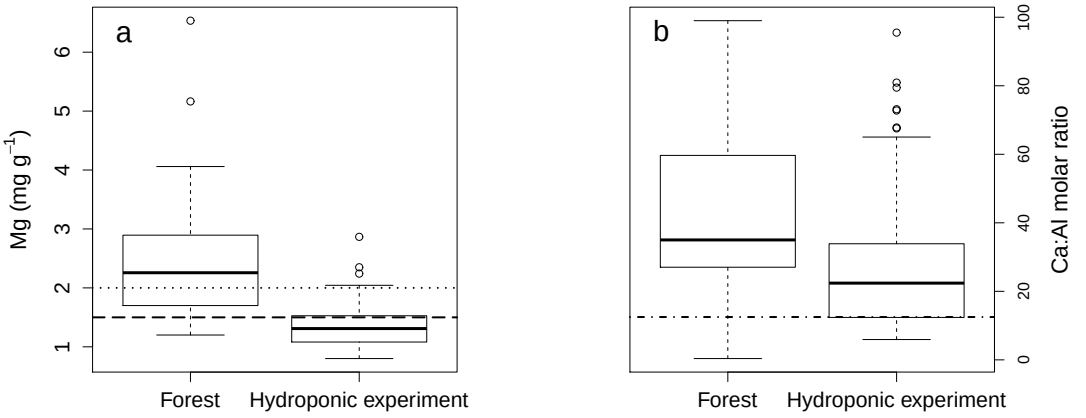

Figure 1.3: Magnesium concentrations (a) and Ca:Al molar ratios (b) in tree leaves from the Ecuadorian forest and the hydroponic experiment in treatments with $600\mu M$ Al and greater. The dotted and the dashed lines (a) are thresholds for optimum growth of crop plants given by Amberger (1996) and Marschner (2012), respectively. The dot-dashed line (b) is the Ca:Al molar ratio in foliar tissue of 12.5 suggested for a 50 % risk of adverse impacts on tree growth induced by Al stress (Cronan and Grigal 1995). Ca:Al molar ratios greater than 100 (7 %) were excluded for illustration purposes

In the studied south Ecuadorian rain forest, high Al fluxes with litterfall attributable to both, high Al concentrations and high quantity of litterfall and the wide distribution of Al-accumulating tree species (Homeier 2008) imply a pronounced Al circulation through the ecosystem. Concluding, Al toxicity in the south Ecuadorian ecosystem cannot be entirely excluded. However, indicators like the generally low plant available free Al^{3+} concentrations in organic layer leachate and mineral soil solution (maximum $< 4\ \mu M$ Al, Fig. 1.2 a), high mean Ca^{2+}:Al^{3+} molar ratios in organic layer leachate above the threshold for Al toxicity (Fig. 1.2 b), and high mean Ca:Al molar ratios in tree leaves (Fig. 1.3 b) from the study site indicate that the plant species are well adapted to the local Al availability and an acute Al toxicity is unlikely.

1.3.6 Error discussion

Laboratory experiments like the hydroponic growth experiment take place under controlled conditions. In this study, I compared an experiment with tree seedlings in nutrient solution with a field observation with mature trees growing in organic horizons and a solid soil matrix, with changing soil moisture, pH values, and periodic acid inputs from the atmosphere. As a consequence, plant available Al concentrations and nutrients in organic layer leachate and soil solutions can vary strongly. In addition, the tree physiology depends on age (Croft et al. 2014) and may differ between mature trees and tree seedlings. Thus, I am aware that a direct comparison of results obtained by laboratory experiments with in-situ conditions in the forested ecosystem is difficult. Nevertheless, even though the effective concentrations (EC values) of Al determined in the hydroponic experiment cannot be seen as absolute significant values applicable to the forest ecosystem, they can help to estimate an acute threat by Al toxicity.

The nutrient solutions for the hydroponic experiment were prepared weekly as 15-L solution in 20-L canisters for the nine replicates of the three tree species together, to ensure equal initial conditions within each treatment. However, there was no calibrated flask of this volume available and the 15-L ultrapure water for the nutrient solution had to be measured with a 3-L beaker. This leads to a weekly variation in element concentrations which was, however, similar for all seedlings

within each weekly treatment. To estimate the error, the coefficient of variation (CV) of Al concentrations in the nutrient solutions (before the treatments) of the six consecutive weeks was calculated for each treatment separately (LL and 0 – 2400 μM), the CV of the other elements was calculated for all treatments of the six weeks together: < 6 % for Al (except control treatment 90 %), < 5 % for Mg, K, Mn, and Ca, < 10 % for Fe, Mo, and total N, < 20 % for Ni and Zn, and 21 %, 52 %, 63 %, and 27 % for Cu, PO_4^{3-}, TP and TOC, respectively. Because the organic layer leachate was prepared each week with a fresh sample of the organic horizons from the study site and the nutrient concentrations were low, already small changes in nutrient composition caused a high CV of up to 100 %. The high CV of Al concentrations in the control treatments results from a contamination with up to 2.5 μM Al. Only in two of the six weekly solutions of the control treatment, the Al concentrations were below detection limit. The Al contamination might originate from the field laboratory in which the nutrient solutions were prepared and where also soil samples (Al-rich dust) and aluminum foil for different sampling procedures are handled. Contamination by metal extraction from canisters and bottles is unlikely, because all canisters and bottles used for the hydroponic experiment were washed in an acid bath (10 % HNO_3) at least over night and rinsed afterwards with ultrapure water.

The bottles in which tree seedlings were placed were covered with black plastic foil to avoid algae growth and additionally with white plastic foil to avoid heating of the nutrient solution. Nevertheless, algae growth occurred in some cases and algae were removed if possible. At harvest, the roots were prewashed thoroughly with tapwater until all algae were removed. At the beginning of the experiment, several problems occurred with the compressor for the aeration. The first compressor broke down in the first week and during its repair the nutrient solutions were not aerated for two days. However, this can be considered as unproblematic as all samples were similarly affected and plant growth was not obviously disturbed. When the compressor broke down the second time in the fifth week it was immediately replaced. Yet, it turned out that the new compressor pumped oil in the aeration hose and a fuel filter with a filter paper had to be interconnected to prevent nutrient solutions from contamination.

The plant material from the hydroponic experiment was washed prior to chemical analysis with ultrapure water, the leaf samples from the forest were not. Thus, a dust cover might contribute to the measured element concentrations, e.g. Al-rich dust. However, dry deposition of Al was negligible compared to total Al fluxes in litterfall and hence, Al concentrations in digests must originate from the leaves themselves rather than Al dust.

To cope with spatial variability regarding nutrient concentrations on plot scale in the studied forest, 20 throughfall collectors, three zerotension lysimeters, and three suction lysimeters were installed (Wullaert 2009). To avoid evaporation, a table-tennis ball was placed in each funnel of the throughfall and bulk precipitation collectors. The bottles used for sampling of throughfall and bulk precipitation were covered with aluminum foil to reduce algae growth. However, the highest Al concentrations and fluxes were determined in litterfall and soil solutions, which were not in contact with any Al objects. Thus, any contamination originating from the Al foil was negligible. The lysimeters were introduced from the wall of soil pit to minimize disturbance of the organic layer. The lysimeters were pushed ca. 0.50 m into the soil so that they were entirely covered by the organic layer. To avoid adsorption/desorption effects in soil solution, a vacuum was applied on the suction cups after installation allowing for equilibration of the cups and the soil solution of the first month was discarded. The sample collectors in the field were not poisoned to prevent biological activity. Thus, chemical transformations might have occurred between the sampling intervals. However, the chemical composition of weekly and daily sampled solutions was not significantly different (Boy 2009).

The quality of plant digestions and analysis of K, Ca, Mg, P, Fe, Mn, Zn, Cu, and Al in plant digests was controlled with the certified reference material (CRM) BCR-100 (beech leaves, IRMM, Geel, Belgium). The accuracy of P, K, Ca, Mn, and Al was within ± 5 %, while that of Mg, Fe, Zn, and Cu was within 10 % of the certified values. The precision of the method was tested by repeated digestion and analysis of samples and reference materials and was within 10 %, except for Al and Cu, which ranged up to 11 and 13 % (RSD), respectively. However, no reference values were available for Ni and Co and therefore those results should be treated with caution. Nevertheless, even if accuracy could not be tested, an adequate precision of the data is sufficient for internal comparison of the different

Al treatments. The detection limits of elements discussed in the Chapters 2, 3, and 4 are given in Table 1.1. The detection limits of elements discussed in the Chapter 5 are given in Boy (2009) and Wullaert (2009). The detection limits of chemical analyses of leaves from the experimental forest sampled in 2005/2006 can be assumed to be similar to those described in Wullaert (2009), as the same analytical devices were used.

Table 1.1: Detection limits of respective elements

element	analytical device	detection limit	unit
Al	ICP-MS	1.25	μg L^{-1}
Ca	AAS	0.021	mg L^{-1}
Cl	CFA	0.10	mg L^{-1}
Co	ICP-MS	0.008	μg L^{-1}
Cu	ICP-MS	0.110	μg L^{-1}
F	ion sensitive electrode	4.68	μg L^{-1}
Fe	ICP-MS	0.073	μg L^{-1}
K	AAS	0.011	mg L^{-1}
Mg	AAS	0.005	mg L^{-1}
Mn	ICP-MS	0.021	μg L^{-1}
Mo	ICP-MS	0.011	μg L^{-1}
Na	AAS	0.009	mg L^{-1}
NH_4^+-N	CFA	0.005	mg L^{-1}
Ni	ICP-MS	0.053	μg L^{-1}
NO_3^--N	CFA	0.052	mg L^{-1}
P	ICP-MS	3.88	μg L^{-1}
o-PO_4-P	CFA	0.005	mg L^{-1}
SO_4^{2-}	IC	0.05	mg L^{-1}
TN	CFA	0.031	mg L^{-1}
TP	CFA	0.005	mg L^{-1}
Zn	ICP-MS	0.159	μg L^{-1}

AAS: atomic absorption spectroscopy
CFA: continuous flow analyzer
IC: ion chromatography
ICP-MS: inductively coupled plasma mass spectrometry

1.4 General conclusions

The results of my research allow to draw the following conclusions:

i) In the hydroponic experiment, aboveground biomass production of all three tree species was strongly reduced by increasing Al concentrations. At 300 μM Al, the root biomass production of *T. chrysantha* was stimulated. The highly productive short-lived pioneer *H. americanus* was more susceptible to Al toxicity than the two old-growth forest tree species *C. odorata* and *T. chrysantha* **(Chapter 2)**.

ii) Of all mineral nutrients, only Mg concentrations in tree leaves were below the thresholds for nutrient deficiency, leading to reduced photosynthesis and reduced biomass production. Enhanced root biomass production of *T. chrysantha* in the treatment with 300 μM Al was most likely the result of stimulated P uptake **(Chapter 3)**.

iii) The good agreement of modeled and measured concentrations of free Al species in soil solutions confirms the suitability of modeling approaches to assess Al speciation in DOM-rich soil solutions. Total dissolved Al concentrations as well as Al^{3+} concentrations were lowest in the organic layer leachate. The free Al^{3+} concentrations steadily increased from organic layer leachate to mineral soil solution in 0.30 m soil depth, which is mainly attributable to decreasing DOC concentrations. Calcium amendment of the forest decreased Ca:Al molar ratios in organic layer leachate because of increasing Al concentrations while Ca concentrations did not change significantly. The enhanced Al concentrations in LL might be caused by decreased Al uptake of plants because of higher Ca availability or exchange of Al^{3+} by Ca^{2+} at soil exchange sites and root surfaces **(Chapter 4)**.

iv) High Al fluxes in the litterfall point at a substantial Al circulation through the ecosystem. Yet, in the key solution for nutrient supply to the plants, i.e. the organic layer leachate, Al concentrations were distinctly below EC10 values determined in the hydroponic experiment and Ca:Al molar ratios

were ten times the threshold suggested for 50 % risk of Al stress. Therefore, Al toxicity is unlikely in the studied ecosystem **(Chapter 5)**.

1.5 Author contributions

I conducted the hydroponic experiment with the supervision of Wolfgang Wilcke and Moritz Bigalke. I carried out the analyses of root and shoot morphology after introduction by Rainer Rees Mertins and Bernd Felderer (ETH Zürich) and all chemical analyses concerning the hydroponic experiment, the analysis of Al concentrations in organic layer leachate from 2003 to 2007, and the sampling and analyses of tree foliage from the study area (sampling in October 2011) after introduction by Daniela Fischer, Michael Wendler, Hanspeter Dettling, Adrien Mestrot, Martin Schwarz, and Moritz Bigalke. Furthermore, I conducted all statistical calculations in the **Chapters 2, 3, and 5**. I wrote the manuscripts **Chapters 1, 2, 3, and 5** and contributed to the manuscript **Chapter 4**, which was written by Moritz Bigalke. The chemical analyses in **Chapter 4** were carried out by Andre Velescu, Sabri Bouanani, and Moritz Bigalke. Martin Schwarz carried out the statistical analyses in **Chapter 4**. Julio Mora Castillo provided the tree seedlings used in the hydroponic experiment **(Chapters 2, 3)**. As a PhD student, Jens Boy was in charge of the Ecuador project within the first five years (1998 – 2003) and performed all related work as well as the calculation of Al loss as particulate matter in streamwater **(Chapter 5)**. Carlos Valarezo was the Ecuadorian partner who contributed to conducting the long-term ecosystem study, facilitated the local work and provided local background knowledge to our experiments. Wolfgang Wilcke evolved the research hypotheses. All authors commented on the respective manuscripts.

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2. Aluminum toxicity to tropical montane forest tree seedlings in southern Ecuador: response of biomass and plant morphology to elevated Al concentrations

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Chapter 2 is published in Plant and Soil 2014, Vol 382, pp. 301–315, DOI 10.1007/s11104-014-2110-0

2.1 Abstract

Aims In acid tropical forest soils (pH < 5.5) increased mobility of aluminum might limit aboveground productivity. Therefore, we evaluated Al phytotoxicity of three native tree species of tropical montane forests in southern Ecuador.
Methods An hydroponic dose-response experiment was conducted. Seedlings of *Cedrela odorata* L., *Heliocarpus americanus* L., and *Tabebuia chrysantha* (Jacq.) G. Nicholson were treated with 0, 300, 600, 1200, and 2400 μM Al and an organic layer leachate. Dose-response curves were generated for root and shoot morphologic properties to determine effective concentrations (EC).
Results Shoot biomass and healthy leaf area decreased by 44% to 83% at 2400 μM Al, root biomass did not respond (*C. odorata*), declined by 51% (*H. americanus*), or was stimulated at low Al concentrations of 300 μM (*T. chrysantha*). EC10 (i.e. reduction by 10%) values of Al for total biomass were 315 μM (*C. odorata*), 219 μM (*H. americanus*), and 368 μM (*T. chrysantha*). *Heliocarpus americanus*, a fast growing pioneer tree species, was most sensitive to Al toxicity. Negative effects were strongest if plants grew in organic layer leachate, indicating limitation of plant growth by nutrient scarcity rather than Al toxicity.
Conclusions Al toxicity occurred at Al concentrations far above those in native organic layer leachate.

2.2 Introduction

Aluminum phytotoxicity is known to occur in acid soils (Alleoni et al. 2010; Delhaize and Ryan 1995; Kochian et al. 2004; Schaedle et al. 1989) and is discussed to be one of the major reasons for limited aboveground biomass productivity and low nutrient-cycling rates in tropical montane forests (Bruijnzeel 2001; Bruijnzeel and Veneklaas 1998; Hafkenscheid 2000; Leuschner et al. 2007). At soil pH values < 5.5, Al is plant-available and phytotoxic as Al^{3+}, $AlOH^{2+}$, or $AlOH_2^+$ and in various inorganic complexes (Kabata-Pendias and Pendias 2001; Macdonald and Martin 1988). For economic reasons, many studies on Al toxicity have been

conducted on crop plants (Kochian et al. 2004; Ryan et al. 2011) and tree species used for afforestations (Kinraide 2003; Schaedle et al. 1989). However, only few tropical forest tree species have been tested for their susceptibility to Al toxicity (Cuenca et al. 1990; Watanabe et al. 1998).

Negative effects of high Al availability include, for instance, inhibition of water and nutrient uptake and plant growth, at which particularly root biomass production is negatively affected (Delhaize et al. 2012; Kochian 1995; Routet al. 2001; Schaedle et al. 1989; Thornton et al. 1987). Inhibition of root elongation is suggested to be a result of Al stress to the root meristem (Ryan et al. 1993). Additionally, under limiting conditions such as root damage or nutrient stress, the plants assign carbohydrates to roots rather than to plant shoots (Harris 1992; Leuschner et al. 2007). Furthermore, exudation of chelating compounds to counteract Al toxicity is energy consuming and happens at the cost of shoot growth (Cuenca et al. 1990). In contrast to these findings, enhancement of root growth (Hajiboland et al. 2013) or plant growth on the whole (Watanabe et al. 1998) has been reported for some tree species at low to moderate Al concentrations in nutrient solution.

At our study site in southern Ecuador, a wide distribution of Al-accumulating plant species has been observed (Homeier 2008), which according to Chenery (1948) have Al concentrations > 1000 mg kg^{-1} in leaves. Predominantly, the Al accumulators appertain to the families Rubiaceae and Melastomataceae (Homeier 2008). Aluminum accumulation is usually interpreted as adaptation to high plant-available Al concentrations (Jansen et al. 2002) and thus Al toxicity.

Processes that increase soil acidification in Ecuadorian montane rain forest, for example fire-derived acid deposition (Boy et al. 2008) and nitrogen input in the form of NH_4^+ (Galloway et al. 2004; Wilcke et al. 2013), might increase Al availability in the future. The increased Al availability may expose native tree species to a greater risk of Al stress. It can furthermore be expected that the fast growing short-lived pioneer tree species are more susceptible to Al toxicity than the more slowly growing long-lived pioneer tree species.

Currently the pH value in the leachate solution below the thick organic layers of the Ecuadorian montane rain forest ranges between 4.6 and 5.2, below the critical value of 5.5 for Al mobilization. Total Al concentrations in these solutions

are low ($< 60\ \mu M$) and $> 97\%$ of Al in the leachate occurs in nontoxic organo-complexes (Wilcke et al. 2001; Wullaert et al. 2013).

Common and economically important tree species in the montane rain forests of southern Ecuador are *Cedrela sp* (Meliaceae) and *Tabebuia chrysantha* (Jacq.) G. Nicholson (Bignoniaceae), which are long-lived pioneers with slow growth rates (Homeier 2008). They belong to the mid-successional tree species and are typically present in primary old-growth forests (Mosandl and Günter 2008). A representative of early-successional species is *Heliocarpus americanus* L. (Tiliaceae), a light demanding fast-growing short-lived pioneer tree (Homeier 2008). These three tree species are not Al accumulators.

In order to test Al toxicity to non-accumulating tropical montane rain forest trees in southern Ecuador, an hydroponic experiment with seedlings of *C. odorata*, *H. americanus*, and *T. chrysantha* was conducted. Our objectives were to:

i) investigate the response of biomass and morphology of native tree species to Al stress,

ii) estimate the sensitivity of the seedlings of native tree species to Al toxicity using dose-response curves, thereby assessing whether fast-growing pioneer tree species or old-growth forest tree species are more susceptible to Al toxicity and

iii) compare growth of tree seedlings under nutrient-optimized conditions to growth in native organic layer leachate.

We hypothesize that:

i) Native tree species in the tropical montane rain forest in southern Ecuador respond negatively to in-vitro elevated Al concentrations and root morphological properties are more strongly affected than shoot morphological properties.

ii) Highly productive pioneer tree species are more susceptible to Al toxicity than slow-growing tree species.

iii) Critical Al concentrations for toxicity in native tree species are above plant-available Al concentrations in organic layer leachate.

2.3 Materials & Methods

2.3.1 Experimental Design

We set up a hydroponic growth experiment with tree seedlings in a greenhouse at the research station San Francisco (4° 00‘ S, 79° 05‘ W), located in the Reserva Biológica San Francisco on the eastern slope of the Cordillera Real, southern Ecuador. Tree seedlings selected for the experiment were *C. odorata*, *H. americanus*, and *T. chrysantha*, aged 7, 6, and 3 months, respectively. These tree species are currently tested as native alternative species for afforestation to replace the locally common exotic *Pinus sp.* and *Eucalyptus sp.* (Mosandl and Günter 2008). Tree seedlings were raised in a nursery from seeds collected from the local forest and germinated in a 50% soil-sand mixture. At the start of the hydroponic experiment, roots were prewashed thoroughly with tap water to remove soil and rinsed with distilled water before placed in nutrient solution. Before addition of Al, tree seedlings were grown for two weeks in nutrient solution.

One tenth Hoagland solution (Hoagland and Arnon 1950) was used as the basis for the experiments because it resembles the nutrient composition of the organic layer leachate of the study area (Tab. 2.1). Nine replicate seedlings per species were treated with 0, 300, 600, 1200, and 2400 μM Al, which was added as $AlCl_3$ to the nutrient solution. The pH was adjusted to 4 using NaOH and HCl. In addition, to simulate plant growth in natural organic layer leachate, one set of replicates of each tree species was treated with an organic layer leachate, without addition of Al. The organic layer leachate used in the experiment was prepared by irrigating a homogenized fresh sample of the whole organic layer from the local forest with distilled water (Tab. 2.1). Each tree seedling was treated with 0.5 L culture solution.

Table 2.1: Nutrient composition of made organic layer leachate and Hoagland nutrient solution

	organic layer leachate	Hoagland nutrient solution
Macronutrients (μM)		
N	281 ± 58.5	1934 ± 29.7
P	10.4 ± 2.8	115 ± 12.1
K	258 ± 56.9	601 ± 3.8
Ca	36.9 ± 6.6	391 ± 2.7
Mg	98.3 ± 21.7	102 ± 0.9
Micronutrients (nM)		
Fe	1486 ± 572	857 ± 15.1
Mn	666 ± 200	198 ± 1.07
Ni	29.6 ± 5.1	60.1 ± 2.0
Cu	122 ± 10.6	94.9 ± 3.6
Zn	133 ± 19.5	252 ± 7.3
Mo	0.36 ± 0.21	51.9 ± 0.75
TOC (nM)	2825 ± 451	24.7 ± 1.2
Al (μM)	44.0 ± 11.3	0 - 2400

Values are means ± SE of solutions before weekly replacement, except for Al in nutrient solution where range of the treatment is given

Pots were placed at random and positions changed weekly, when culture solutions were replaced and sampled (mixed sample of the nine replicates per species and treatment). Nutrient solutions were aerated for 15 minutes per hour to ensure aerobic conditions at all times. After six weeks, plants were harvested, washed thoroughly with distilled water and leaves, stem, and roots were separated. Leaves were scanned immediately after separation and dried in a drying oven at 55°C to constant weight. Roots were stored cool until scanning with a root scanner and dried at 55°C to constant weight immediately after scanning. In addition to the dry weight of roots and shoots (stem and leaves combined), the fresh weight of the total plants before and after the experiment (growth rate) was determined. Of the 162 seedlings in total, only 4 plants died during the experiment (two seedlings of each of *H. americanus* and *T. chrysantha*, i.e. one seedling of each species in each of the 0 and 2400 μM treatments, respectively) and were removed without replacement. Leaves were scanned at 300 dpi (24 bit) in colour with a Canon scanner (CanoScan LiDE 100). Roots were scanned with an Epson Expression 10000 XL at 600 dpi (8 bit) grey scale. The scanner was equipped with additional lighting system in the lid to avoid distortion by shadows. Analysis of healthy and diseased leaf area, total root length, average root diameter, root volume, root tips, and root forks was carried out with WinRhizo 2009 (Regent Instruments Inc., Canada).

2.3.2 Chemical analyses

Roots, stems, and leaves were separated and 50 mg plant material were digested in a microwave oven (MLS Ethos, Germany). To ensure dissolution of aluminosilicates a digestion with 1.6 mL 69% HNO_3 , 0.6 mL 30% H_2O_2, 0.1 mL 48% HF, and 1 mL 5% H_3BO_3 was chosen. Aluminum concentrations in digests of plant tissue were determined with ICP-MS 7700X (Agilent Technologies, Germany). Calcium concentrations were determined with AAS Zeenit 700P (Analytik Jena, Germany). Total nitrogen and total organic carbon (TOC) in nutrient solution were analyzed with a Vario TOC Cube (Elementar Analysensysteme, Germany). NH_4^+-N, NO_3^--N, and PO_4^{3-}-P concentrations in organic layer leachate before and

after weekly treatment were analyzed with a Continuous Flow Analyzer (CFA AutoAnalyzer 3 HR , SEAL Analytical, Germany).

2.3.3 Statistical analyses

A Principal Component Analysis (PCA, R, package psych, Revelle 2013) with varimax rotation was applied in order to reduce the number of variables and to extract interrelations among the root and shoot parameters. The PCA was conducted for the three tree species combined and included the variables healthy and diseased leaf area, shoot and root biomass (dry weight), root-to-shoot biomass ratio, total root length, root diameter, root volume, root tips, and root forks. As an important morphological property which is considered indicative of toxicity effects, the root-to-shoot biomass ratio was included in addition to root and shoot biomass (Graham 2001).

Differences among treatments were tested using one-way ANOVA and post-hoc tests. When ANOVA residuals were normally distributed (Shapiro-Wilk normality test) and showed homogeneity of variances (Bartlett test of homogeneity of variances), as post-hoc test Fisher's least significant difference (LSD) test with Bonferroni correction was chosen for *C. odorata* and Tukey's honest significant difference (HSD) test for unequal n for *H. americanus* and *T. chrysantha*, because of the loss of two replicates of each of these species. When normal distribution and homogeneity of variances could not be assumed, the Games-Howell test was used. Differences in concentrations of NH_4^+-N, NO_3^--N, and PO_4^{3-}-P in organic layer leachate before and after weekly treatment were tested with the Mann-Whitney U test. Significance was set at $p < 0.05$ unless otherwise indicated; ** denotes $p < 0.01$ and *** $p < 0.001$.

Dose-response curves (DRC) were fitted using log(x+1)-transformed Al concentrations in nutrient solutions vs. total biomass (dry weight), healthy leaf area, number of root tips, root diameter, root-to-shoot biomass ratio and diseased leaf area (in % of total leaf area). For the number of root tips a function could only be fitted for *H. americanus*. Fitting failed for root diameter of *T. chrysantha*, for root-to-shoot biomass ratio of *H. americanus*, and for diseased leaf area of *C. odorata* (Fig. 2.5).

Effective concentrations (EC) were calculated for 10, 20, and 50% reduction or enhancement, respectively, compared to control. The treatment with organic layer leachate was considered as a supplemental experiment and was excluded from ANOVA, PCA, and DRC fitting, because strong differences in chemical composition of the solutions complicated a comparison. Statistical analyses were carried out mainly with R 2.13.1 for Windows GUI front-end (R Foundation, Austria). For the Games-Howell test SPSS 19 (IBM Corp., United States) was used. Dose-response curves were fitted with Origin 8.5 for Windows (OriginLab Corporation, USA).

2.4 Results

2.4.1 Principal component analysis of plant properties

Three principal components (PC) were extracted. The first PC was highly loaded by properties characteristic for biomass (Tab. 2.2). The second PC was highly loaded by properties related to root morphology. Root diameter was negatively related to all other root parameters. The third PC was highly loaded by plant properties which are known for their particular susceptibility to Al toxocity, i.e. diseased leaf area and root-to-shoot biomass ratio. All plant properties were significantly affected by increasing Al concentrations with few exceptions (Tab. 2.3).

Biomass related plant properties Net total plant growth (difference in fresh weight of the whole plant between start and harvest, not included in the PCA) decreased with increasing Al concentrations by 49%, 77%, 46% for *C. odorata*, *H. americanus*, and *T. chrysantha*, respectively, and was smallest in the treatment with organic layer leachate. Shoot biomass decreased as Al concentrations increased to 2400 μM by 44%, 73%, 56% for *C. odorata*, *H. americanus*, and *T. chrysantha*, respectively (Fig. 2.1, Tab. 2.3). *H. americanus* showed the most distinct decrease of shoot biomass, reflected in the slope of the regressions (β) of shoot biomass on Al concentrations: β = -2.8 * 10^{-4}, r = -0.54***; β = -5.8 * 10^{-4}, r = -0.73***; β = -3.2 * 10^{-4}, r = -0.60*** for *C. odorata*, *H. americanus*, and *T. chrysantha*, respectively.

Table 2.2: Principal component (PC) loadings of plant biomass and morphological properties

	PC1	PC2	PC3
healthy leaf area	**0.91**	0.11	-0.25
diseased leaf area	-0.03	0.39	**0.63**
shoot biomass	**0.89**	0.36	-0.16
root biomass	**0.88**	0.21	0.34
root-to-shoot biomass ratio	0.07	-0.13	**0.88**
total root length	0.52	**0.82**	0.08
root diameter	0.46	**-0.73**	0.40
root volume	**0.86**	-0.02	0.40
root tips	0.18	**0.95**	0.13
root forks	0.26	**0.93**	0.07
explained variance	0.37	0.33	0.17

The data was varimax rotated and only PCs with Eigenvalues > 1 were accepted. Loadings > 0.6 are bold

Root biomass was not significantly different among the treatments for *C. odorata*, and decreased significantly by 51% for *H. americanus* as Al concentrations increased (Fig. 2.1, Tab. 2.3). For *T. chrysantha* root biomass was significantly higher by 68% at 300 μM Al compared to control, and decreased to near the control value at 2400 μM Al. This pattern suggests a stimulation in root biomass production of *T. chrysantha* at low Al concentrations.

Similar to shoot biomass, healthy leaf area decreased for all tree species with increasing Al concentration to 2400 μM by 51%, 83%, 53%, for *C. odorata*, *H. americanus*, and *T. chrysantha*, respectively (Tab. 2.3). Again, a regression of leaf area on Al concentrations showed the most distinct gradient in decrease of healthy leaf area for *H. americanus* (slope of regression: β = -0.22, r = -0.57***; β = -0.35, r = -0.81***; β = -0.19, r = -0.64*** for *C. odorata*, *H. americanus*, and *T. chrysantha*, respectively).

Root morphology Morphological root properties of *H. americanus* responded most markedly to increasing Al concentrations (Fig. 2.2). The number of root tips was not significantly different among the Al treatments for *C. odorata* and *T. chrysantha*, yet in *H. americanus* it decreased significantly by 58% at 2400 μM Al. The root diameter of *C. odorata* and *H. americanus* increased with increasing

Table 2.3: Net total plant growth (g fresh weight), shoot and root biomass (g dry weight), root-to-shoot biomass ratio, healthy leaf area (cm^2), deseased leaf area (% of total leaf area), total root length (cm), root diameter (mm), root volume (cm^3), forks, and tips for *C. odorata*, *H. americanus*, *T. chrysantha* after 7 weeks of treatment with Hoagland nutrient solution containing 0, 300, 600, 1200, and 2400 μM Al and organic layer leachate

	Al concentration (μM)					
	0	300	600	1200	2400	organic layer leachate
Net plant growth (g)						
C. odorata	11.3 ± 1 a	10.5 ± 1.20 ab	7.80 ± 0.57 abc	7.16 ± 0.71 bc	5.80 ± 0.39 c	1.24 ± 0.06
H. americanus	14.2 ± 0.87 a	13.8 ± 1.02 a	7.10 ± 1.03 b	6.99 ± 0.72 b	3.60 ± 0.78 b	2.62 ± 0.11
T. chrysantha	8.10 ± 0.66 ab	9.02 ± 0.82 a	6.21 ± 0.41 bc	6.07 ± 0.48 bc	4.37 ± 0.40 c	0.68 ± 0.14
Shoot biomass (g)						
C. odorata	1.71 ± 0.12 a	1.48 ± 0.16 ab	1.17 ± 0.11 bc	1.07 ± 0.12 bc	0.96 ± 0.07 c	0.30 ± 0.04
H. americanus	2.11 ± 0.16 a	1.67 ± 0.16 a	1.01 ± 0.14 b	0.88 ± 0.09 b	0.56 ± 0.10 b	0.31 ± 0.03
T. chrysantha	1.40 ± 0.10 b	1.40 ± 0.10 a	0.87 ± 0.10 c	0.89 ± 0.09 bc	0.61 ± 0.06 c	0.22 ± 0.03
Root biomass (g)						
C. odorata	0.61 ± 0.06	0.68 ± 0.08	0.49 ± 0.07	0.54 ± 0.07	0.46 ± 0.04	0.13 ± 0.02
H. americanus	0.65 ± 0.07 ab	0.70 ± 0.06 a	0.39 ± 0.06 bc	0.40 ± 0.06 bc	0.32 ± 0.08 c	0.12 ± 0.01
T. chrysantha	0.28 ± 0.04 b	0.47 ± 0.05 a	0.31 ± 0.02 ab	0.34 ± 0.04 ab	0.24 ± 0.03 b	0.06 ± 0.01
Root-to-shoot biomass ratio						
C. odorata	0.35 ± 0.02 b	0.45 ± 0.02 a	0.42 ± 0.03 ab	0.51 ± 0.03 a	0.47 ± 0.01 a	0.41 ± 0.04
H. americanus	0.30 ± 0.02	0.42 ± 0.02	0.40 ± 0.04	0.48 ± 0.08	0.52 ± 0.07	0.41 ± 0.06
T. chrysantha	0.20 ± 0.01 b	0.32 ± 0.01 a	0.38 ± 0.02 ab	0.38 ± 0.02 a	0.39 ± 0.02 a	0.32 ± 0.08
Healthy leaf area (cm^2)						
C. odorata	1207 ± 119 a	985 ± 110 ab	794 ± 70 b	705 ± 82 b	597 ± 45 b	144 ± 10
H. americanus	1075 ± 63 a	924 ± 59 a	605 ± 98 b	528 ± 56 b	187 ± 39 b	155 ± 4.9
T. chrysantha	866 ± 77 a	848 ± 88 a	603 ± 49 ab	548 ± 50 b	410 ± 46 b	79 ± 11
Diseased leaf area (% of total)						
C. odorata	10 ± 4.0	8.2 ± 2.3	12 ± 1.7	12 ± 2.7	12 ± 3.0	11 ± 1.5
H. americanus	11 ± 1.2 bc	8.9 ± 1.2 c	12 ± 1.1 bc	18 ± 2.3 b	54 ± 4.5 a	15 ± 1.3
T. chrysantha	2.6 ± 0.24 b	2.9 ± 0.34 b	6.7 ± 2.0 ab	8.3 ± 1.4 a	13 ± 2.1 a	19 ± 1.7
Total root length (cm)						
C. odorata	1033 ± 74 a	1086 ± 110 a	756 ± 52 ab	795 ± 58 ab	638 ± 39 b	421 ± 24
H. americanus	1967 ± 118 a	2065 ± 108 a	1319 ± 158 b	1202 ± 93 b	840 ± 139 b	950 ± 38
T. chrysantha	960 ± 80 a	946 ± 76 a	737 ± 31 ab	795 ± 72 ab	644 ± 50 b	203 ± 39
Root diameter (mm)						
C. odorata	0.68 ± 0.02 ab	0.67 ± 0.01 b	0.72 ± 0.02 ab	0.71 ± 0.02 ab	0.76 ± 0.02 a	0.51 ± 0.01
H. americanus	0.42 ± 0.02 b	0.43 ± 0.02 b	0.48 ± 0.03 ab	0.54 ± 0.02 a	0.51 ± 0.03 ab	0.32 ± 0.01
T. chrysantha	0.48 ± 0.02 b	0.55 ± 0.02 a	0.56 ± 0.01 a	0.54 ± 0.02 a	0.48 ± 0.02 b	0.53 ± 0.02
Root volume (cm^3)						
C. odorata	3.83 ± 0.34	3.87 ± 0.46	3.11 ± 0.37	3.24 ± 0.36	2.88 ± 0.23	0.86 ± 0.06
H. americanus	2.75 ± 0.33	3.17 ± 0.41	2.65 ± 0.42	2.87 ± 0.39	1.88 ± 0.42	0.80 ± 0.08
T. chrysantha	1.76 ± 0.24 ab	2.32 ± 0.31 a	1.80 ± 0.07 a	1.80 ± 0.13 a	1.19 ± 0.14 b	0.43 ± 0.07
Root forks						
C. odorata	1340 ± 171 ab	1527 ± 235 a	1011 ± 114 ab	847 ± 115 ab	722 ± 77 b	405 ± 81
H. americanus	8505 ± 784 a	8801 ± 1039 a	4098 ± 470 b	3548 ± 247 b	2532 ± 488 b	2430 ± 115
T. chrysantha	1956 ± 175 a	1784 ± 235 ab	1275 ± 96 b	1464 ± 197 ab	1255 ± 168 ab	284 ± 51
Root tips						
C. odorata	459 ± 43	614 ± 76	445 ± 37	519 ± 48	464 ± 50	250 ± 24
H. americanus	3466 ± 224 a	3114 ± 275 a	1792 ± 194 b	1674 ± 140 b	1467 ± 231 b	1203 ± 72
T. chrysantha	587 ± 32	723 ± 65	575 ± 43	808 ± 81	646 ± 94	202 ± 28

Data refer to means of 9 replicates (8 replicates for *H. americanus* and *T. chrysantha* for the treatments with 0 and 2400 μM Al) ± SE. Lower case letters depict significant differences among treatments with Hoagland nutrient solution at $p < 0.05$

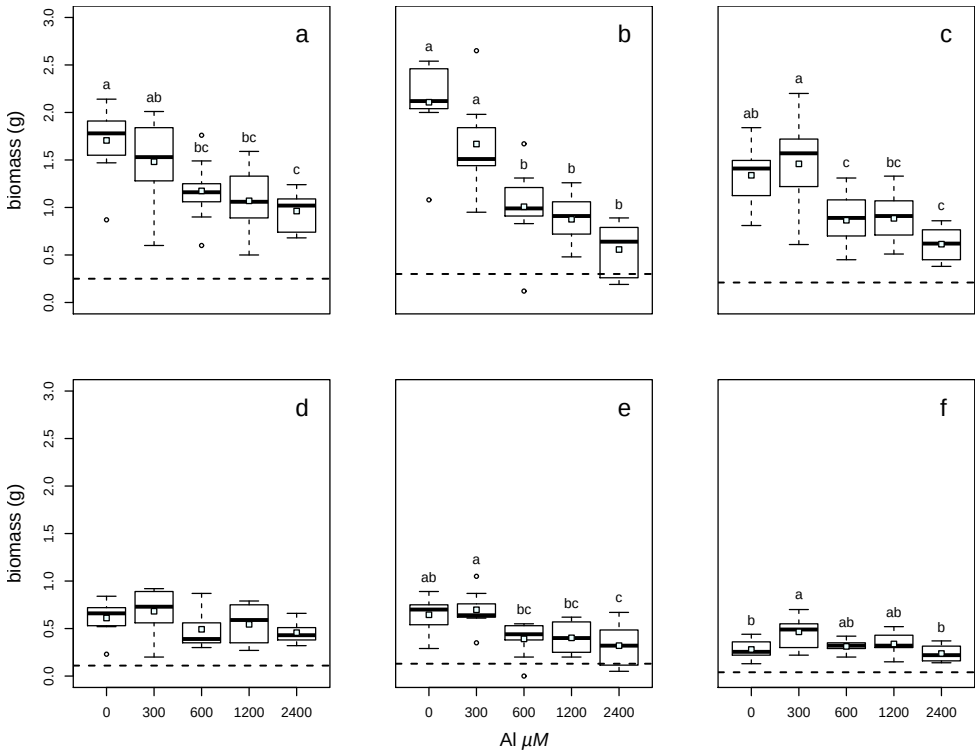

Figure 2.1: Boxplots of aboveground (upper row) and root (lower row) biomass for *C. odorata* (a, d), *H. americanus* (b, e), and *T. chrysantha* (c, f) by treatment (0, 300, 600, 1200, and 2400 μM Al). Black bars represent the median, whiskers represent the minimum-maximum range of the group data. Group means are given as white squares. Lower case letters above the boxplots depict significant differences among the treatments at $p < 0.05$. The dashed line shows the median in treatment with organic layer leachate

Al concentration (by 12% and 21% at 2400 μM, respectively). Root diameter of *T. chrysantha* increased by 17% at 600 μM Al and decreased at further increasing Al concentrations to similar values like in the control (Tab. 2.3).

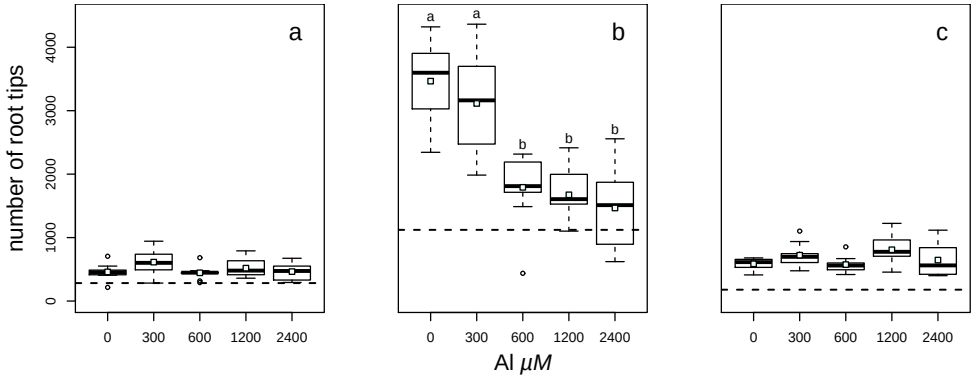

Figure 2.2: Boxplots of the number of root tips for *C. odorata* (a), *H. americanus* (b), and *T. chrysantha* (c) by treatment (0, 300, 600, 1200, and 2400 μM Al). Black bars represent the median, whiskers represent the minimum-maximum range of the group data. Group means are given as white squares. Lower case letters above the boxplots depict significant differences among the treatments at $p < 0.05$. The dashed line shows the median in treatment with organic layer leachate

Indications of Al toxicity There was a significant negative correlation between number of root tips and root diameter for all tree species combined (r = -0.82***) and individually for *H. americanus* (r = -0.93**). Root-to-shoot biomass ratio increased significantly with Al concentration for *C. odorata* and *T. chrysantha* by 34% and 95% at 2400 μM Al, resulting from a more pronounced decrease in shoot biomass compared to root biomass (Fig. 2.3, Tab. 2.3).

We observed diseased areas of pale green, yellow, and brown colors on the leaves (Fig. 2.4). The diseased leaf area in % of total leaf area was not significantly different among the Al treatments for *C. odorata*. However, the fraction of the deseased leaf area increased from 11±1.2% and 2.6±0.24% in the control treatment to 54±4.5% and 13±2.1% in the treatment with 2400 μM Al in *H. americanus* and *T. chrysantha*, respectively (Tab. 2.3).

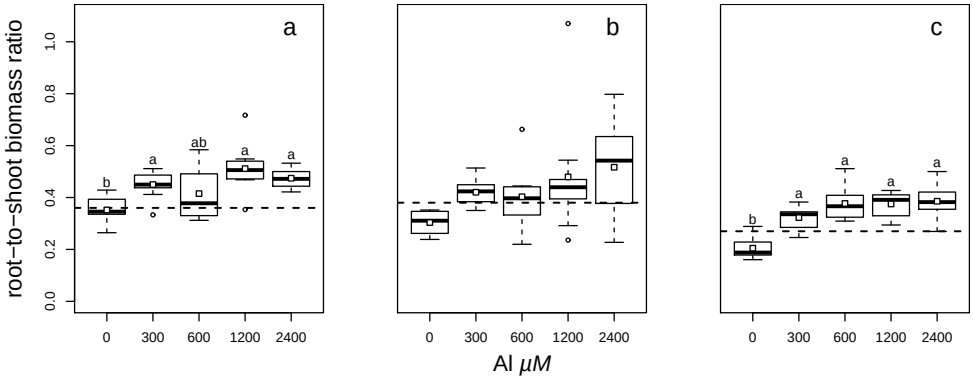

Figure 2.3: Boxplots of root-to-shoot biomass ratio for *C. odorata* (a), *H. americanus* (b), and *T. chrysantha* (c) by treatment (0, 300, 600, 1200, and 2400 μM Al). Black bars represent the median, whiskers represent the minimum-maximum range of the group data. Group means are given as white squares. Lower case letters above the boxplots depict significant differences among the treatments at $p < 0.05$. The dashed line shows the median in treatment with organic layer leachate

2.4.2 Dose-response curves

Best fits for all tree species were achieved for the relationship between Al concentrations and total biomass and healthy leaf area, respectively. The EC10 values, i.e. the effective concentration of Al at which the respective plant property was affected by 10% relative to the control, for all tree species and all fitted plant properties (total biomass, healthy leaf area, number of root tips, root diameter, and diseased leaf area in % of total) ranged from 150 to 1736 μM, EC20 from 299 to 726 μM, and EC50 from 475 to 2271 μM. The EC10 values of root-to-shoot biomass ratio ranged from 5.48 to 182 μM, the EC20 values from 38.3 to 212 μM, and the EC50 value of *T. chrysantha* was 281 μM. The root-to-shoot biomass ratio appeared to be the most Al-sensitive plant property, with *C. odorata* responding far more sensitively than *T. chrysantha* (Tab. 2.4). With respect to total biomass reduction at increasing Al concentrations, *H. americanus* responded most sensitively, while *C. odorata* and *T. chrysantha* had similar EC values. Ten and 20% reduction of healthy leaf area occurred first in *C. odorata*, followed by *H. americanus* and *T. chrysantha*. With respect to root diameter enhancement, *H. americanus* responded more sensitively to increasing Al concentrations than

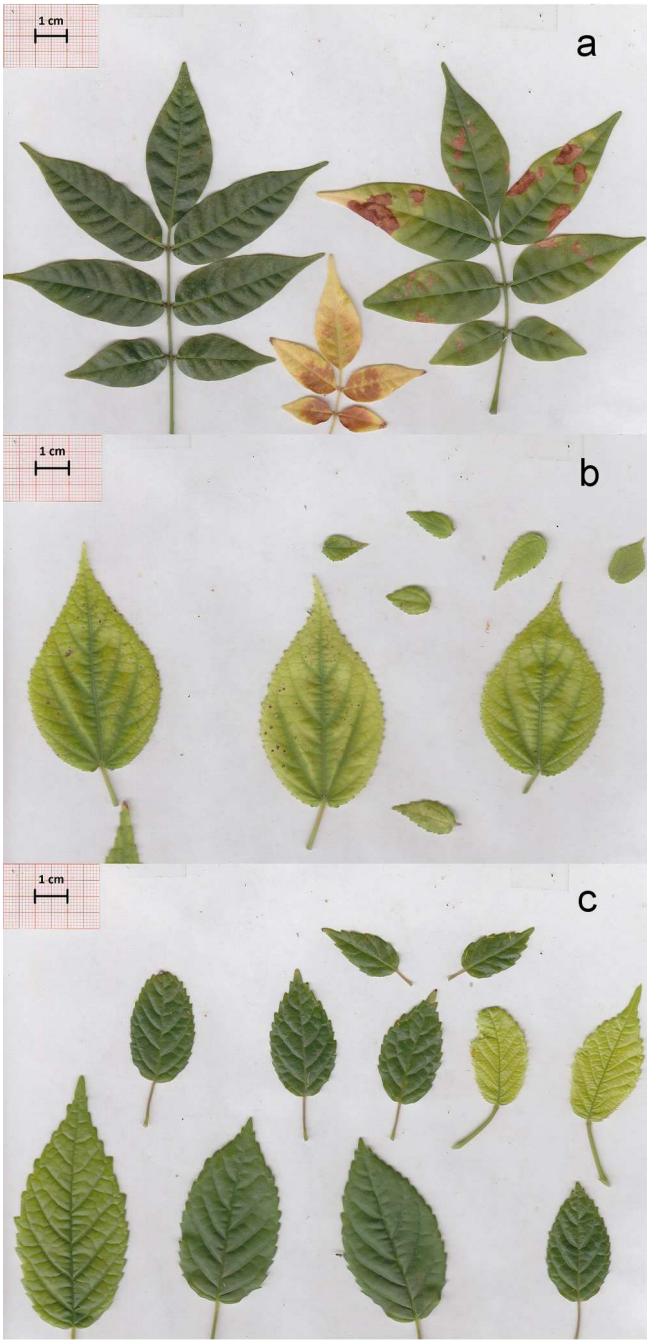

Figure 2.4: Diseased leaves of *C. odorata* (a), *H. americanus* (b), and *T. chrysantha* (c) in the 2400 μM Al treatment. The picture gives a qualitative example of the pale green, yellow, and brown colors of diseased spots.

C. odorata. Except for the response of diseased leaf area in *C. odorata*, either *H. americanus* or *C. odorata* responded most sensitively to increasing Al concentrations (Fig. 2.5, Tab. 2.4).

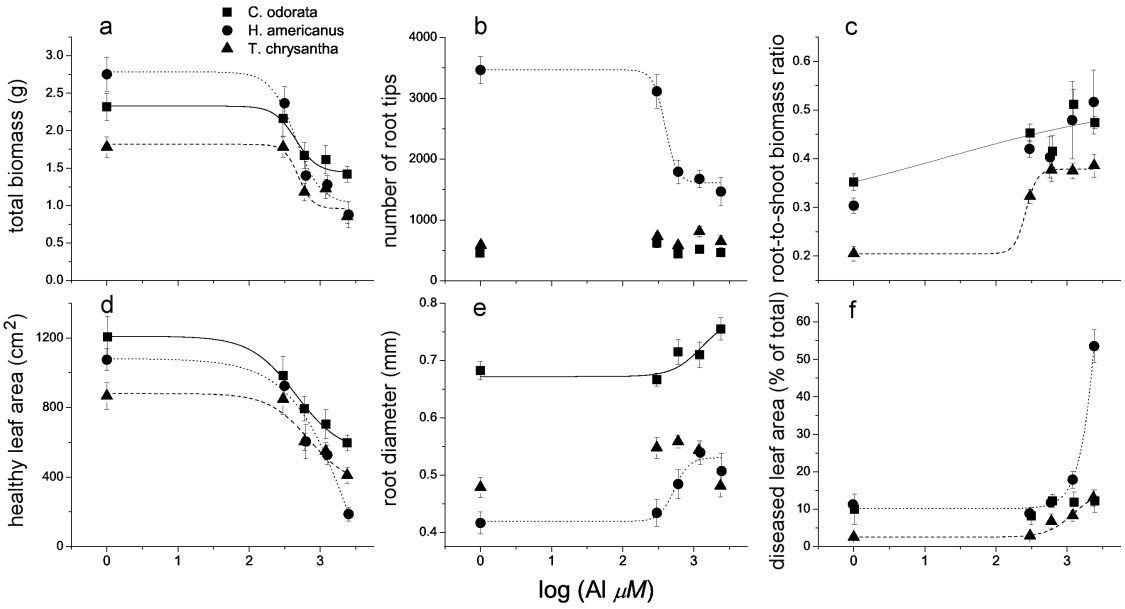

Figure 2.5: Dose-response curves of plant properties to Al concentrations for total biomass (dry weight) (a), number of root tips (b), root-to-shoot biomass ratio (c), healthy leaf area (cm^2) (d), root diameter (mm) (e), and diseased leaf area (% of total) (f), for *C. odorata*, *H. americanus*, and *T. chrysantha*, respectively. Error bars represent SE of means. Lines are fitted sigmoid growth functions

2.4.3 Chemical plant properties

Aluminum concentrations in roots were significantly higher in the treatments with Al than in the control treatments (means±SE for *C. odorata*, *H. americanus*, and *T. chrysantha*: 1.42±0.30, 0.00±0.00, and 0.35±0.14 mg g^{-1}). Among the treatments with 300 to 2400 μM Al no significant differences were observed for *C. odorata* and *T. chrysantha* (mean Al concentrations ±SE over all Al treatments: 14±0.6 and 6.2±0.4 mg g^{-1}, respectively). Only for *H. americanus*, Al concentrations in roots increased significantly from 9.2±0.7 mg g^{-1} at 300 μM Al to 19±0.8 mg g^{-1} at 2400 μM Al.

Table 2.4: EC10, EC20, and EC50 values (in μM Al, i.e. effective Al concentrations, at which 10, 20, and 50% reduction or enhancement compared to control occurs) for total biomass, healthy leaf area, number of root tips, root diameter, root-to-shoot biomass ratio, and diseased leaf area (% of total) of *C. odorata*, *H. americanus*, *T. chrysantha*

Parameter	*C. odorata*	*H. americanus*	*T. chrysantha*
Total biomass			
EC10	315	219	368
EC20	467	315	461
EC50	-	733	-
Healthy leaf area			
EC10	150	163	241
EC20	299	350	440
EC50	2271	1093	2001
Number of root tips			
EC10	-	299	-
EC20	-	359	-
EC50	-	657	-
Root diameter			
EC10	1736	473	-
EC20	-	693	-
EC50	-	-	-
Root-to-shoot biomass ratio			
EC10	5.48	-	182
EC20	38.3	-	212
EC50	-	-	281
Diseased leaf area (% of total)			
EC10	-	563	229
EC20	-	726	311
EC50	-	1017	475

The hyphen (–) indicates that no EC values could be determined

The Ca concentrations in leaves (mean±SE for *C. odorata*, *H. americanus*, and *T. chrysantha* in control/2400 μM Al, respectively: 19±0.4/7.5±0.6, 14±0.5/6.7±0.7, 11±0.5/9±0.8 mg g^{-1}) were significantly different among the treatments. In *C. odorata* and *H. americanus* they first decreased from control to treatment with 300 μM Al, increased at 600 μM Al and decreased again with further increasing Al treatment. In *T. chrysantha* the mean Ca range was high in leaves treated with 300 μM Al and then decreased in treatments with 600 and 1200 μM Al and increased again in the highest Al treatment.

The Ca:Al molar ratios in leaf tissue decreased significantly from control to treatment with 2400 μM Al in all tree species (Fig. 2.6). In root tissue, Ca:Al molar ratios of all plants were distinctly higher in control than in all other treatments (Fig. 2.6).

The TOC concentrations in nutrient solution after weekly treatment increased significantly in the treatments above 300 μM Al (Fig. 2.7).

2.4.4 Organic layer leachate experiment

The mean of shoot and root biomass (Fig. 2.1), healthy leaf area, and number of root tips (Fig. 2.2) for all tree species was lower in the treatment with organic layer leachate than in control and all Al treatments (Tab. 2.3). The mean root diameter of *C. odorata* and *H. americanus* was lower in the organic layer leachate than in control and all Al treatments. The mean of root-to-shoot biomass ratios of the seedlings of all species grown in organic layer leachate was higher than that of the control but lower than the mean of all Al treatments (Tab. 2.3). The mean diseased leaf area (in % of total leaf area) of plants grown in organic layer leachate exceeded that of all other treatments for *T. chrysantha* and the majority of the other treatments for *C. odorata* and *H. americanus* (Tab. 2.3).

Mean Ca concentrations in leaves of plants treated with organic layer leachate were lower than in plants of the control treatment and ranged among the Al treatments.

The TOC concentrations in organic layer leachate after weekly treatment were with 32.8±6.4, 32.4±5.4, and 30.6±5.5 mg L^{-1} (mean±SE for *C. odorata*, *H. americanus*, and *T. chrysantha*, respectively) almost 20 times higher

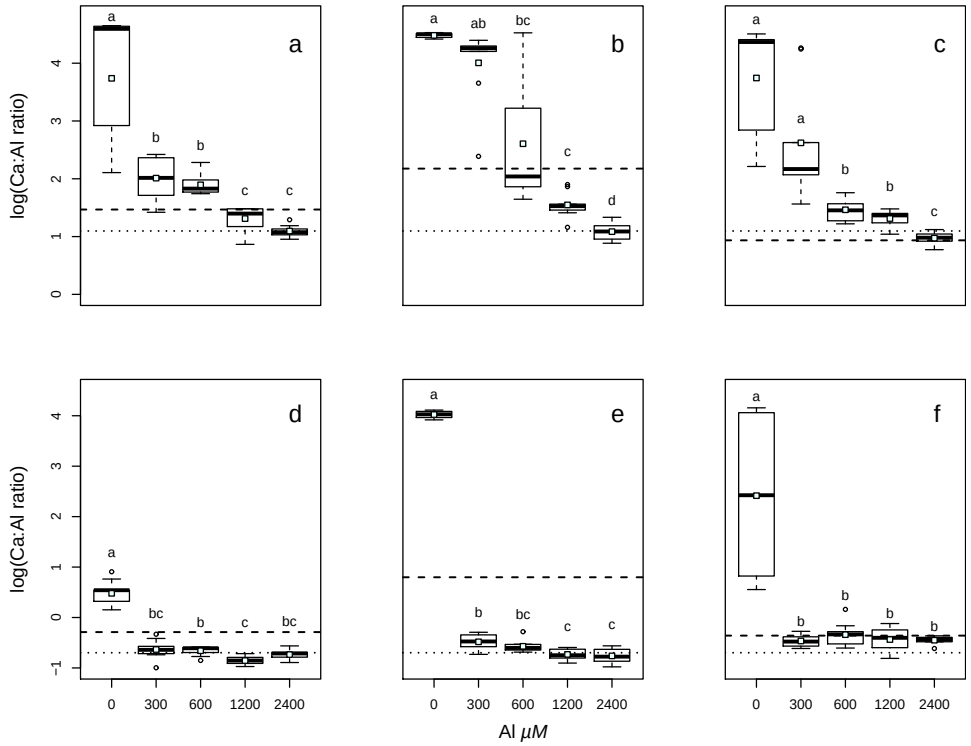

Figure 2.6: Boxplots of log-transformed Ca:Al molar ratios in leaf tissue (upper row) and root tissue (lower row) of *C. odorata* (a, d), *H. americanus* (b, e), and *T. chrysantha* (c, f) by treatment (0, 300, 600, 1200, and 2400 μM Al). Black bars represent the median, whiskers represent the minimum-maximum range of the group data. Group means are given as white squares. Lower case letters above the boxplots depict significant differences among the treatments at $p < 0.05$. The dashed line shows the median in treatment with organic layer leachate. The dotted line shows critical Ca:Al molar ratios as Al-stress indicators for leaves and roots, respectively (Cronan and Grigal 1995)

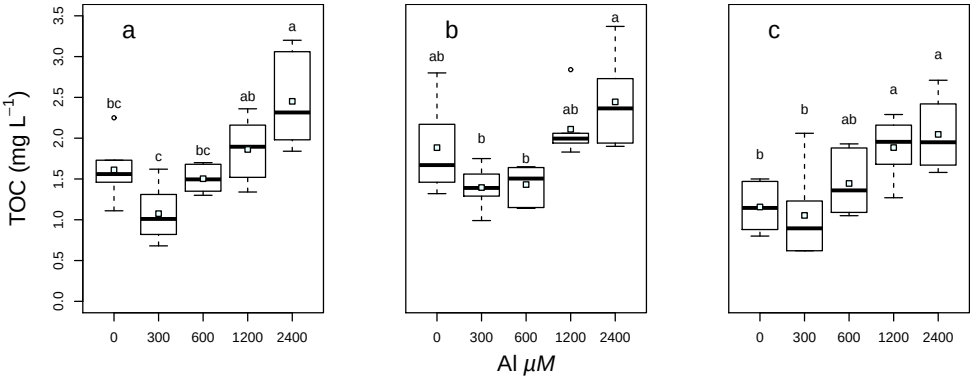

Figure 2.7: Boxplots of TOC concentrations (mg L^{-1}) in nutrient solution after weekly treatment of *C. odorata* (a), *H. americanus* (b), and *T. chrysantha* (c) by treatment (0, 300, 600, 1200, and 2400 μM Al). n = 6, *black bars* represent the median, *whiskers* represent the minimum-maximum range of the group data. Group means are given as *white squares*. Lower case letters above the boxplots depict significant differences among the treatments at < 0.05

than in Hoagland nutrient solution. The concentrations of NH_4^+-N in organic layer leachate decreased during the weekly treatments significantly for *C. odorata* and *H. americanus* from 1.65±0.3 mg L^{-1} to 0.6±0.2 and 0.4±0.1 mg L^{-1}, respectively. The concentrations of NO_3^--N and PO_4^{3-}-P in organic layer leachate decreased significantly in the course of the experiment, illustrating substantial nutrient depletion by plant uptake for *H. americanus* only (from 1.1±0.5 and 0.2±0.08 mg L^{-1} to 0.1±0.08 and 0.03±0.01 mg L^{-1}, respectively), but not for the other two species.

2.5 Discussion

2.5.1 Aluminum effects on plant biomass and morphology

Several plant properties were negatively affected by increased dissolved Al concentrations. For most properties, there was no significant difference between control and treatment with 300 μM Al, as well as among treatments with 600, 1200, and 2400 μM Al, suggesting that Al concentrations of 300 μM must be exceeded to induce significant negative effects on biomass and morphology (Figs. 2.1 and

2.2). Significant reduction of plant biomass at Al concentrations of 250 μM was reported for red spruce (*Picea rubens* Sarg.) seedlings by Thornton et al. (1987). The reason for lacking plant responses to low Al concentrations may be related with efficient defense mechanisms against Al effects. Some of the mechanisms counteracting Al stress are release of chelating agents like citrate, malate, and oxalate from the root apex which form non-toxic Al-complexes, or phosphate, that increases pH of the rhizosphere and precipitates insoluble compounds which cannot be taken up by plants (Brunner and Sperisen 2013; Hafkenscheid 2000; Pellet et al. 1997, 1995). The increasing TOC concentrations in nutrient solutions of the high Al treatments suggest that the plants responded to Al stress by the release of chelating compounds (Fig. 2.7).

Surprisingly, aboveground plant properties (shoot biomass and leaf area) responded more sensitively to Al stress than root properties (Fig. 2.1 and 2.2). While shoot biomass decreased by 44 to 73% compared to control, root biomass either remained unchanged across the treatments (*C. odorata*), declined (*H. americanus*) or was even stimulated al low Al concentrations of 300 μM and then decreased again at higher Al concentrations (*T. chrysantha*). This pattern shows that Al was not interacting directly with the root apex but possibly disturbed nutrient uptake.

The decrease of Ca concentrations in leaf tissue might result from an inhibited long distance transport of Ca, induced by Al blocking Ca channels (Huang et al. 1992; Rengel 1992), which contributes to a negative shoot biomass response to elevated Al concentrations. In *T. chrysantha* the Ca decrease was less pronounced than in the other two species, indicating less blocking of Ca transport, which is in line with the less negative response of aboveground biomass production compared to the other two tree species. Compared to other tree species from the same study site, however, even the lowest Ca concentration in leaves was high. Wilcke et al. (2008) reported for *Graffenrieda emarginata* (Ruiz & Pav.), an Al-accumulating plant species, Ca concentrations in leaves of 1.2-3.7 mg g^{-1}. In a Ca fertilizing experiment in the Ecuadorian montane forest (Wullaert et al. 2013) trees from control plots had Ca concentrations in leaves ranging from 0.82±0.3 mg g^{-1} (*Myrcia* sp.nov.) up to 3.25±1.05 mg g^{-1} (*Alchornea lojaensis* Secco). Our results demonstrate that the increasing Al concentrations in the solution indeed reduced

Ca concentrations in leaves suggesting that reduced Ca translocation in the plant contributed to decreased biomass production. However, even at the highest Al concentration in solution, Ca concentrations in leaves did not drop below those found in the forest.

According to Cronan and Grigal (1995) Ca:Al molar ratios of < 12.5 and < 0.2 in leaf and root tissue, respectively, can be used as threshold values indicating Al stress to forest trees. In our experiment, Ca:Al molar ratios in leaves of all three plant species only approached the threshold value of 12.5 in the highest Al treatment but did not reach a value below this threshold. However, in the treatment with organic layer leachate, *T. chrysantha* showed a molar Ca:Al ratio below 12.5 suggesting Al stress (Fig. 2.6). In root tissue of *C. odorata* and *H. americanus* the Ca:Al molar ratios in all Al treatments were close to the threshold value for roots. In *T. chrysantha* Ca:Al molar ratios in roots were in all treatments above the threshold. Wullaert et al. (2013) reported Ca:Al molar ratios in leaves of tree species native to the Ecuadorian montane forest, ranging from 0.31 ± 0.07 in the Al-accumulating *Graffenrieda emarginata* (Ruiz & Pav.) to 71 in *Hieronyma fendleri* Briq. We attribute the high Ca:Al molar ratios to the growth of the seedlings in a particularly Ca-rich substrate in the tree nursery.

Root-to-shoot biomass ratios increased (Fig. 2.3), contrasting some results in the literature. Thornton et al. (1987) found decreasing root-to-shoot ratios for red spruce seedlings, treated with Al concentrations as high as 2000 μM. Other studies, however, are more consistent with our findings. Graham (2001) reported a negative effect of 1000 μM Al on peach seedlings (*Prunus persica* (L.) Batsch) in sand culture, reducing number and length of plant lateral shoots, total shoot growth, leaf number, and leaf area, but not root, stem, or leaf dry weight. Kidd and Proctor (2000) investigated the Al tolerance of birch populations (*Betula pendula* Roth) from different ecological sites in a culture solution experiment. In some populations Kidd and Proctor (2000) discovered an enhancement in plant growth at 74 - 185 μM Al, followed by growth inhibition at Al concentrations > 370 μM. Growth of an Al-sensitive population from a calcareous soil was inhibited at all Al concentrations in solution (74 - 1300 μM). In contrast, growth of Al-tolerant populations increased with increasing Al concentrations up to 926 μM in solution.

Furthermore, a recent study by Hajiboland et al. (2013) revealed stimulation of root growth in a tea plant (*Camellia sinensis* (L.) Kuntze) at 300 μM Al. Beneficial effects in culture solution for both, root and shoot biomass, were found for pine (*Pinus radiata* D.Don) and eucalypt (*Eucalyptus mannifera* Mudie subsp. *mannifera*) seedlings, whereas strongest plant growth of eucalypt occurred at 2222 μM Al and of pine at 370 μM Al (Huang and Bachelard 1993). The beneficial effects of Al on plant growth are explained by alleviation of H^+ toxicity at low pH values, which is ascribed to promoted H^+ extrusion and increase of cell membrane electrical polarity (Kinraide 1993). The resulting electrochemical gradient induces a stimulation of nutrient uptake (Osaki et al. 1997).

In our experiment, the number of root tips was unaffected in *C. odorata* and *T. chrysantha*, but reduced by 60% in *H. americanus*. This pattern is in line with the Al concentrations in roots, which were not significantly different among the Al treatments for *C. odorata* and *T. chrysantha* but increased for *H. americanus* with increasing Al cocentrations in the nutrient solution. The higher sensitivity of *H. americanus* can be explained by the fact that the number of root tips in the control was 6 to 7 times higher than of the other two species, illustrating a highly dispersed root architecture, which might be particularly vulnerable to Al stress (Tab. 2.3).

2.5.2 Sensitivity to Al exposure

Schaedle et al. (1989) classified sensitive species with growth effects at Al concentrations below 150 μM, which include honeylocust (*Gleditsia triacanthos* L.), coffee (*Coffea arabica* L.), white spruce (*Picea glauca* Voss), and peach (*Prunus persica* (L.) Batsch), intermediately sensitive species responding between 150 and 800 μM Al which include sugar maple (*Acer saccharum* Marsch.), red (*Picea rubens* Sarg.) and black spruce (*Picea mariana* Mill.), European beech (*Fagus sylvatica* L.) and loblolly pine (*Pinus taeda* L.), and resistant species which only respond to Al concentrations above 800 μM. To directly compare the response of our studied tree species with those in the review of Schaedle et al. (1989), we calculated the EC10 values for aboveground biomass, as aboveground biomass was highly susceptible to Al toxicity. According to the classification by Schaedle

et al. (1989) under in-vitro conditions, the light-demanding fast-growing short-lived pioneer species *H. americanus* (EC10 126 μM) can be classified as sensitive species, and the long-lived pioneers *C. odorata* (EC10 238 μM) and *T. chrysantha* (EC10 376 μM) as intermediately sensitive species.

2.5.3 Hoagland nutrient solution versus organic layer leachate

Plants grew generally worse and showed more damages when grown in organic layer leachate than in treatments with Hoagland nutrient solution, irrespective of Al concentration. Accordingly, the highly complex matrix of the organic layer leachate, i.e. nutrient scarcity and possibly complexation of essential micronutrients by organic compounds, must pose problems influencing plant growth more than dissolved Al.

Although pH values in the organic layer leachate were below 5.5, mean total Al concentrations in solution were low ($< 44 \pm 11$ μM, Tab. 2.1) and $> 97\%$ were generally bound in organo-Al-complexes (Wullaert et al. 2013), which are known to mask and detoxify Al. At present, our results indicate limitation of biomass production of *C. odorata*, *H. americanus*, and *T. chrysantha* by other factors than Al phytotoxicity in the organic layer leachate. The most likely reason are the low concentrations of nutrients (Tab. 2.1). This explanation would be in line with findings of Homeier et al. (2012), who recently reported limitation of aboveground productivity in the studied area by simultaneous N and P scarcity. Accordingly, plant-available N and P concentrations in the organic layer leachate tended to decrease during the treatment. However, the reduction of NH_4^+-N was only significant for *C. odorata* and *H. americanus*. The reduction of NO_3^--N and PO_4^{3-}-P was only significant for *H. americanus*, indicating that either N and P uptake is complicated by other factors for *C. odorata* and *H. americanus* or that other nutrients which we did not consider (e.g. B) could also play a role in limitation of aboveground biomass productivity of the tropical montane forests in southern Ecuador.

2.6 Conclusions

i) Aluminum stress caused negative effects on root and shoot morphology of *C. odorata*, *H. americanus*, and *T. chrysantha* above 150 μM of dissolved Al. Yet, shoot properties were stronger affected than root properties.

ii) The short-lived pioneer *H. americanus* was most sensitive to Al toxicity confirming that highly productive pioneer tree species could be considered as more vulnerable to Al stress than old-growth forest tree species.

iii) Based on the Al concentrations in organic layer leachate which fall below critical Al concentrations detected in our experiment, there are no indications for an important role of Al toxicity at our study site.

2.7 Acknowledgments

We thank Karoline Klaus, Katharina Kitzinger, Jose Luis Peña Caivinagua, and Orly Mendoza Aguirre for support in set up of the experiment, Rainer Rees Mertins and Bernd Felderer, ETH Zurich, CH, for support with WinRhizo, Nature and Culture International in Loja, EC, for providing the study area and the research station, Ecuadorian authorities for the research permit, and the German Research Foundation (DFG) for funding (FOR 816, Wi 1601/8-2).

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3. Aluminum toxicity to tropical montane forest tree seedlings in southern Ecuador: response of nutrient status to elevated Al concentrations

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Chapter 3 is published online in Plant and Soil 2014, pp 1–11, DOI 10.1007/s11104-014-2276-5

3.1 Abstract

Aims We determined the reasons why in nutrient solution increasing Al concentrations $> 300\ \mu M$ inhibited shoot biomass production of *Cedrela odorata* L., *Heliocarpus americanus* L., and *Tabebuia chrysantha* (Jacq.) G. Nicholson while $300\ \mu M$ Al stimulated root biomass production of *Tabebuia chrysantha*.
Methods Nutrient concentrations in plant tissue after a hydroponic growth experiment were determined.
Results Increasing Al concentrations significantly decreased Mg concentrations in leaves. Phosphorus concentrations in roots of *C. odorata* and *T. chrysantha* were significantly highest in the treatment with $300\ \mu M$ Al and correlated significantly with root biomass.
Conclusions Shoot biomass production was likely inhibited by reduced Mg uptake, impairing photosynthesis. The stimulation of root growth at low Al concentrations can be possibly attributed to improved P uptake.

3.2 Introduction

The soils of the tropical montane rain forests in southern Ecuador have pH values < 5.5 resulting in high Al concentrations in soil solution. Therefore, Al phytotoxicity was discussed to be a major reason for limited aboveground biomass production and for a slow nutrient cycling (Bruijnzeel 2001; Bruijnzeel and Veneklaas 1998; Hafkenscheid 2000; Leuschner et al. 2007). The currently observed soil acidification in tropical montane forests of southern Ecuador because of forest fire derived acids (Boy et al. 2008) and increasing NH_4^+ deposition with subsequent nitrification and NO_3^- leaching (Wilcke et al. 2013) might enhance Al toxicity in future.

A hydroponic Al-toxicity experiment with three selected native tree species from southern Ecuador showed a negative impact of Al concentrations $> 300\ \mu M$ in nutrient solution on shoot biomass production, healthy leaf area and root properties of *Cedrela odorata* L., *Heliocarpus americanus* L., and *Tabebuia chrysantha* (Jacq.) G. Nicholson. However, these concentrations were higher than usual

concentrations in organic layer leachate ($< 60\ \mu M$) (Rehmus et al. 2014). Surprisingly, the root biomass production was not (*C. odorata* and *T. chrysantha*) or only marginally affected (*H. americanus*) by elevated Al concentrations, and therefore root to shoot biomass ratios increased in response to elevated Al concentrations. Increasing TOC concentrations with increasing Al concentrations in the nutrient solutions after the treatments nevertheless suggested a release of organic acids as a defence mechanism against Al toxicity. Because Ca:Al molar ratios in leaves and roots did not fall below reported threshold values for Al toxicity (< 12.5 in leaves and < 0.2 in fine roots, Cronan and Grigal 1995), the Ca-Al antagonism is unlikely to be a reason for the observed toxicity effects, pointing at the interaction between Al and other nutrients.

Aluminum was reported to reduce concentrations of P and K in Al-sensitive plants (Graham 2001; Osaki et al. 1997; Thornton et al. 1987). Reduced uptake and translocation of Mg was also observed in various studies (Graham 2001; Keltjens 1995; Kinraide 2003; Osaki et al. 1997; Thornton et al. 1987). As the central ion of the chlorophyll molecule, Mg has a crucial role in photosynthesis. Magnesium deficiency leads to depressed plant growth, the translocation of Mg from mature to young leaves and protein degradation, causing chlorosis in mature leaves (Marschner 2012). An impaired nutrient uptake and/or root-shoot translocation and resulting inhibition of physiological processes in the tree seedlings might therefore be the explanation for the reduction in shoot biomass production.

Moreover, in the experiment of Rehmus et al. (2014) enhanced root biomass production was observed for *T. chrysantha* in the treatment with 300 μM Al. Stimulation of plant growth induced by toxic metal ions at low concentrations is known as hormesis (Calabrese and Blain 2009; Poschenrieder et al. 2013). Poschenrieder et al. (2013) distinguish three main mechanisms behind growth stimulation, which are I) amelioration by substrate interactions, II) activation of stress specific defenses like lag-time dependent activation of tolerance mechanisms and priming or acclimation by preexposure to low concentrations of the toxic element, and III) activation of general defense mechanisms, for instance against oxidative and biotic stress. We suppose substrate interactions or amelioration of biotic or oxidative stress as reason for the observed root growth stimulation.

A stimulation of plant growth and especially of P but also N and K uptake by application of Al was observed for some Al-tolerant plant species by Osaki et al. (1997). Hajiboland et al. (2013a) investigated the mechanisms for Al-induced growth stimulation in an Al-tolerant tea plant (*Camellia sinensis* L. Kuntze) in a hydroponic experiment and attributed the growth stimulation to enhanced photosynthesis rates and a better protection of membranes against peroxidation by the activation of antioxidant defense enzymes.

Several studies investigated the P-Al interactions in the context of Al-toxicity resp. Al-tolerance (Jiang et al. 2009; Liao et al. 2006; Watanabe and Osaki 2001; Zheng et al. 2005), suggesting both, alleviation of Al toxicity by precipitation of Al-P compounds in the roots or on the root surface as well as stimulated extrusion of specific organic acids, which chelate the trivalent Al cation.

Our objectives were:

i) to test whether negative effects on shoot biomass production result from suppressed nutrient supply of shoots.

ii) to identify the reason for enhanced root biomass production of *T. chrysantha* at 300 μM Al.

We hypothesize that

i) reduced shoot biomass production with increasing plant available Al concentrations can be explained by inhibited mineral nutrient supply, i.e. N, P, K, and/or Mg supply,

ii) stimulated root growth of *T. chrysantha* at 300 μM Al was caused by elevated N and/or P uptake.

3.3 Materials & Methods

3.3.1 Experimental Design

We set up a hydroponic growth experiment with tree seedlings in a greenhouse at the research station San Francisco (4° 00‘ S, 79° 05‘ W), located in the Reserva

Biológica San Francisco on the eastern slope of the Cordillera Real, southern Ecuador. Tree seedlings selected for the experiment were *C. odorata*, *H. americanus*, and *T. chrysantha*, aged 7, 6, and 3 months, respectively. These tree species are currently tested as native alternative species for afforestation to replace the locally common exotic *Pinus sp.* and *Eucalyptus sp.* (Mosandl and Günter 2008). Tree seedlings were raised in a nursery from seeds collected from the local forest and germinated in a 50 % soil-sand mixture. At the start of the hydroponic experiment, roots were prewashed thoroughly with tap water to remove soil and rinsed with distilled water before placed in nutrient solution. Before addition of Al, tree seedlings were grown for two weeks in nutrient solution.

One tenth Hoagland solution (Hoagland and Arnon 1950) was used as the basis for the experiments because it resembles the nutrient composition of the organic layer leachate of the study area (Rehmus et al. 2014). However, an important dissimilarity to the organic layer leachate is that in our nutrient solution P concentrations were about 10 times higher than in organic layer leachate. This implies a better P supply of the plants grown in hydroponic solution than in the forest soil and possibly a related deterioration of the plant availability of Fe and Zn (Marschner 2012; Poschenrieder et al. 2013). Nine replicate seedlings per species were treated with 0, 300, 600, 1200, and 2400 μM Al, which was added as $AlCl_3$ to the nutrient solution. The pH was adjusted to 4 using NaOH and HCl.

Each tree seedling was treated with 0.5 L culture solution. Pots were placed at random and positions changed weekly, when culture solutions were replaced. Nutrient solutions were aerated for 15 minutes per hour to ensure aerobic conditions at all times. After six weeks, plants were harvested and washed thoroughly with distilled water. Leaves, stems, and roots were separated and dried in a drying oven at 55°C to constant weight. Shoot weight was determined for stems and leaves together. Of the 135 seedlings treated with Al and the control solution, only 4 plants died during the experiment (two seedlings of each of *H. americanus* and *T. chrysantha*, i.e. one seedling of each species in each of the 0 and 2400 μM treatments, respectively) and were removed without replacement.

3.3.2 Chemical analyses

Fifty mg of plant material (roots and leaves) were digested in a closed-vessel microwave system (MLS Ethos, Germany). To ensure dissolution of aluminosilicates a digestion with 1.6 mL 69 % HNO_3 , 0.6 mL 30 % H_2O_2, 0.1 mL 48 % HF, and 1 mL 5 % H_3BO_3 was chosen. Concentrations of P, Fe, Mn, Zn, Cu, Ni, Co, and Al were determined with ICP-MS (7700x Agilent Technologies, Germany). Potassium, Ca, and Mg concentrations were determined with AAS (Zeenit700P Analytik Jena, Germany). Carbon, N, and S concentrations were analyzed with a Vario EL Cube (Elementar Analysensysteme, Germany). If root quantity was not sufficient for both, digestion and CNS analysis, sample digestion was preferred.

The quality of digestions and analysis of K, Ca, Mg, P, Fe, Mn, Zn, Cu, and Al was controlled with the certified reference material (CRM) BCR-100 (beech leaves, IRMM, Geel, Belgium). The accuracy of P, K, Ca, Mn, and Al was within ± 5 %, while that of Mg, Fe, Zn, and Cu was within 10 % of the certified values. The precision of the method was tested by repeated digestion and analysis of samples and reference materials and was always within 10 %, except for Al and Cu, which ranged up to 11 and 13 % (RSD), respectively. No reference values are available for Ni and Co.

3.3.3 Statistical analyses

Differences in nutrient concentrations among treatments were tested using one-way ANOVA and post-hoc tests. When ANOVA residuals were normally distributed (Shapiro-Wilk normality test) and showed homogeneity of variances (Bartlett test of homogeneity of variances), as post-hoc test Fisher's least significant difference (LSD) test with Bonferroni correction was chosen for equal group n and Tukey's honest significant difference (HSD) test for unequal group n. When normal distribution and homogeneity of variances could not be assumed, the Games-Howell test was used. Differences in P concentrations of roots and shoots were tested with Welch's two sample t-test.

To test for correlations between selected variables a Pearson Product Moment Correlation was chosen for variables which showed normal distribution. For variables which did not show normal distribution, a nonparametric Spearman's rank

correlation (r_s) was conducted. Statistical analyses were carried out with R 3.0.2 (R Core Team 2013) and SPSS (SPSS 19 IBM Corp., United States).

3.4 Results

3.4.1 Concentrations of macronutrients in plant tissue

Nitrogen concentrations in roots and leaves of *H. americanus* increased significantly with increasing Al concentrations (Tab. 3.1). In leaves of *C. odorata*, N concentrations increased from the control to 1200 μM Al and dropped at 2400 μM Al to the control value. In roots of *T. chrysantha* treated with 300 μM Al, the mean N concentration was significantly lower than at 600 μM and 2400 μM Al. Mean N concentrations were not significantly different among the Al treatments in leaves of *T. chrysantha* and in roots of *C. odorata*.

The mean P concentrations in leaves of *C. odorata* and *T. chrysantha* were significantly highest in the control although P concentrations in leaves of *T. chrysantha* were not significantly different between the control and the 2400 μM Al treatment (Tab. 3.1). In *H. americanus*, P concentrations in leaves were higher in the control and at 600 and 2400 μM Al than at 300 and 1200 μM Al. In roots of all tree species, mean P concentrations were highest at 300 μM Al. In roots of *C. odorata*, the P concentrations decreased with further increase of Al concentration in nutrient solution. In *H. americanus*, the P concentration first decreased at 1200 μM Al and increased significantly at 2400 μM Al. The P concentrations in roots of *T. chrysantha* were significantly lowest at 600 μM Al and increased with further increasing Al concentration. In general, the P concentrations in the roots were significantly higher than in the leaves. This difference in P concentrations was highest at 300 μM Al, with P concentrations being up to three times higher in roots than in leaves.

Concentrations of K in leaves of all tree species increased significantly with increasing Al concentrations (Tab. 3.1). In roots of all tree species, K concentrations were significantly lowest at 2400 μM Al and not significantly different among the other treatments.

Table 3.1: Concentrations of macronutrients (mg g^{-1}) in leaf and root tissue of *C. odorata*, *H. americanus*, and *T. chrysantha* after 7 weeks of treatment with Hoagland nutrient solution containing 0, 300, 600, 1200, and 2400 μM Al

		Al concentration (μM)				
		0	300	600	1200	2400
N (mg g^{-1})						
C. odorata	leaves	32.2 ± 0.83 bc	36.6 ± 0.86 ab	36.6 ± 1.20 ab	38.4 ± 1.37 a	32.1 ± 1.03 c
	roots	31.9 ± 1.12	32.8 ± 1.05	34.8 ± 0.86	34.2 ± 1.04	35.0 ± 0.88
H. americanus	leaves	26.6 ± 1.3 c	27.8 ± 1.5 c	33.4 ± 0.82 (8) b	36.1 ± 0.81 ab	40.9 ± 1.59 a
	roots	27.9 ± 1.01 d	30.3 ± 1.02 cd	34.7 ± 1.45 (8) bc	38.9 ± 1.19 ab	40.9 ± 1.01 (7) a
T. chrysantha	leaves	35.3 ± 1.06	35.9 ± 0.73	36.9 ± 0.98	36.1 ± 1.19	35.5 ± 0.7
	roots	35.5 ± 1.75 (5) ab	33.9 ± 1.67 b	39.8 ± 1.46 a	40.5 ± 0.63 (5) ab	41.3 ± 1.30 a
P (mg g^{-1})						
C. odorata	leaves	3.92 ± 0.17 a	2.48 ± 0.20 b	2.51 ± 0.20 b	2.58 ± 0.28 b	2.28 ± 0.07 b
	roots	4.83 ± 0.22 b	8.21 ± 1.02 a	6.16 ± 0.41 ab	5.26 ± 0.27 b	5.45 ± 0.4 b
H. americanus	leaves	5.10 ± 0.40 a	1.88 ± 0.13 b	2.63 ± 0.86 ab	2.00 ± 0.09 b	4.21 ± 0.43 a
	roots	6.53 ± 0.47 ab	8.12 ± 0.59 a	6.50 ± 0.89 ab	5.58 ± 0.57 b	7.76 ± 0.27 a
T. chrysantha	leaves	3.17 ± 0.12 a	2.20 ± 0.15 b	2.43 ± 0.19 b	2.26 ± 0.12 b	2.74 ± 0.11 ab
	roots	4.02 ± 0.24 b	5.97 ± 0.24 a	2.90 ± 0.19 c	3.96 ± 0.29 b	4.82 ± 0.32 b
K (mg g^{-1})						
C. odorata	leaves	20.5 ± 0.49 c	25.0 ± 2.08 bc	31.5 ± 1.07 ab	31.3 ± 2.02 ab	33.6 ± 0.88 a
	roots	41.4 ± 1.87 (8) a	40.9 ± 1.81 a	35.9 ± 2.79 ab	36.5 ± 2.53 ab	29.2 ± 0.74 b
H. americanus	leaves	20.7 ± 1.02 c	21.6 ± 1.07 c	27.4 ± 2.73 bc	32.9 ± 1.54 b	47.5 ± 2.53 a
	roots	28.8 ± 1.49 a	29.8 ± 1.81 a	30.3 ± 2.15 a	27.2 ± 1.33 a	17.8 ± 0.77 b
T. chrysantha	leaves	22.3 ± 1.01 b	25.0 ± 1.80 ab	28.1 ± 1.68 ab	27.7 ± 0.98 a	28.2 ± 0.98 a
	roots	33.4 ± 1.09 ab	38.1 ± 1.98 a	38.3 ± 1.95 a	35.2 ± 1.92 a	26.2 ± 1.91 b
Ca (mg g^{-1})						
C. odorata	leaves	19 ± 0.4 a	11.3 ± 0.98 bc	13.6 ± 1.26 b	8.81 ± 0.8 c	7.49 ± 0.57 c
	roots	5.42 ± 0.26 a	4.56 ± 0.17 ab	4.04 ± 0.23 b	2.84 ± 0.12 c	3.97 ± 0.27 b
H. americanus	leaves	13.6 ± 0.47 a	7.84 ± 0.92 bc	11.3 ± 0.91 ab	5.45 ± 0.29 c	6.74 ± 0.68 c
	roots	4.4 ± 0.26	4.37 ± 0.37	4.65 ± 0.64	3.68 ± 0.21	4.79 ± 0.51
T. chrysantha	leaves	10.5 ± 0.45 a	13.7 ± 2.17 ab	6.96 ± 0.43 b	6.46 ± 0.32 b	8.99 ± 0.82 ab
	roots	5.42 ± 0.34 a	3.25 ± 0.08 b	2.99 ± 0.21 b	3.63 ± 0.25 b	3.51 ± 0.28 b
Mg (mg g^{-1})						
C. odorata	leaves	2.72 ± 0.13 a	2.17 ± 0.21 a	1.23 ± 0.04 b	1.15 ± 0.10 b	1.23 ± 0.11 b
	roots	1.79 ± 0.06 a	1.31 ± 0.04 b	0.83 ± 0.03 c	0.76 ± 0.04 c	0.75 ± 0.03 c
H. americanus	leaves	2.15 ± 0.09 a	2.09 ± 0.18 a	1.63 ± 0.16 a	1.06 ± 0.04 b	1.74 ± 0.12 a
	roots	2.57 ± 0.25 a	2.39 ± 0.14 a	1.41 ± 0.06 b	0.97 ± 0.02 c	0.88 ± 0.05 c
T. chrysantha	leaves	2.67 ± 0.14 (7) a	2.33 ± 0.10 a	1.51 ± 0.09 b	1.22 ± 0.07 b	1.36 ± 0.08 b
	roots	2.72 ± 0.09 a	2.22 ± 0.16 a	1.09 ± 0.04 b	1.00 ± 0.05 b	0.72 ± 0.04 c
S (mg g^{-1})						
C. odorata	leaves	2.74 ± 0.08 a	2.71 ± 0.08 a	2.46 ± 0.06 a	2.53 ± 0.09 a	2.14 ± 0.07 b
	roots	3.74 ± 0.33	3.10 ± 0.19	3.11 ± 0.10	3.30 ± 0.11	3.03 ± 0.04
H. americanus	leaves	1.86 ± 0.10 b	1.78 ± 0.06 b	1.88 ± 0.03 (8) b	1.91 ± 0.04 b	2.39 ± 0.12 a
	roots	4.97 ± 0.38 a	3.33 ± 0.20 c	3.76 ± 0.13 (8) bc	4.27 ± 0.19 abc	4.56 ± 0.25 (7) ab
T. chrysantha	leaves	2.70 ± 0.07 a	2.44 ± 0.04 ab	2.28 ± 0.08 bc	2.13 ± 0.07 c	2.21 ± 0.06 bc
	roots	5.09 ± 0.21 (5) a	2.53 ± 0.05 c	2.81 ± 0.08 bc	3.12 ± 0.05 (5) b	3.07 ± 0.13 b

Data refer to means of 9 replicates ± SE and 8 replicates ± SE for *H. americanus* and *T. chrysantha* in the treatments with 0 and 2400 μM Al. If we lost replicates because of insufficient sample quantity or eliminated outliers, numbers of replicates included in the statistical analysis are given in parentheses. Lower case letters depict significant differences among the treatments at $p < 0.05$

In leaves of *C. odorata* and *H. americanus*, the Ca concentrations decreased with increasing Al concentrations while *T. chrysantha* showed no clear effect (Tab. 3.1). In the roots of *C. odorata*, the Ca concentrations significantly decreased from control to 1200 μM Al and increased at 2400 μM Al, while *H. americanus* showed no effect. The Ca concentrations in roots of *T. chrysantha* were significantly highest in the control.

Magnesium concentrations in leaves of *C. odorata* and *T. chrysantha* were significantly higher in the control and at 300 μM Al than at 600, 1200, and 2400 μM Al (Tab. 3.1). In leaves of *H. americanus*, the significantly lowest Mg concentrations were observed at 1200 μM Al. In the roots, the Mg concentrations of all tree species decresased significantly from control to 2400 μM Al.

The mean S concentrations in leaves of *C. odorata* and *H. americanus* were not significantly different up to 1200 μM Al (Tab. 3.1). At 2400 μM Al, the S concentration in leaves of *C. odorata* was significantly lowest but significantly highest in leaves of *H. americanus*. In leaves of *T. chrysantha*, S concentrations significantly decreased from control to 2400 μM Al. There were no significant differences in S concentrations among the treatments in roots of *C. odorata*. In roots of *H. americanus* and *T. chrysantha*, the highest S concentrations occurred in the control, yet they increased from 300 to 2400 μM Al.

3.4.2 Concentrations of micronutrients, beneficial mineral elements, and Al in plant tissue

No consistent patterns which could be attributed to Al impact were found for Mn and Co concentration (data not shown) in leaf and root tissue, although in some cases differences among the treatments were significant.

Significant differences among the treatments were found in mean concentrations of Fe, Zn, Cu, and Ni, partly showing consistent increasing or decreasing patterns which could be attributed to Al exposure (Tab. 3.2).

No significant differences in root Al concentrations were found for *C. odorata* and *T. chrysantha* growing at increasing Al concentrations, while for *H. americanus*, Al concentrations in roots increased significantly (Rehmus et al. 2014).

Table 3.2: Concentrations of micronutrients (μg g^{-1}) in leaf and root tissue and Al concentrations (mg g^{-1}) in leaf tissue of *C. odorata*, *H. americanus*, and *T. chrysantha* after 7 weeks of treatment with Hoagland nutrient solution containing 0, 300, 600, 1200, and 2400 μM Al

		Al concentration (μM)				
		0	300	600	1200	2400
Fe (μg g^{-1})						
C. odorata	leaves	112 ± 15.3 ab	105 ± 18.1 ab	84.4 ± 6.2 b	159 ± 17.9 a	138 ± 5.9 a
	roots	651 ± 100 b	1,517 ± 370 a	796 ± 133 ab	1,156 ± 154 ab	1,070 ± 149 ab
H. americanus	leaves	95.8 ± 14.8	56.4 ± 12.6	80.6 ± 13.9	77.7 ± 8.2	155 ± 62.2
	roots	91.9 ± 11.6	99.6 ± 13.8	211 ± 70.4	192 ± 48.9	179 ± 18.2
T. chrysantha	leaves	95.8 ± 17.8	94.3 ± 19.7	110 ± 8.29	108 ± 7.44	240 ± 71.0
	roots	381 ± 75	375 ± 145	235 ± 71.1	480 ± 137	221 ± 49.6
Zn (μg g^{-1})						
C. odorata	leaves	34.1 ± 1.83	38.3 ± 3.39	34.4 ± 2.38	37.7 ± 4.20	30.3 ± 1.87
	roots	86.7 ± 10.1 a	87.2 ± 11.4 ab	83.3 ± 8.22 a	72.6 ± 10.4 ab	50.8 ± 3.61 b
H. americanus	leaves	28.3 ± 2.45	31.7 ± 2.11	33.0 ± 7.17	23.0 ± 1.45	35.8 ± 4.17
	roots	21.4 ± 1.30 a	22.3 ± 1.64 a	22.4 ± 2.39 ab	15.2 ± 0.78 b	15.0 ± 0.89 b
T. chrysantha	leaves	52.1 ± 4.60 ab	58.2 ± 6.18 a	39.2 ± 2.36 b	38.4 ± 3.19 b	39.5 ± 1.67 b
	roots	58.4 ± 14.6 a	30.3 ± 3.31 b	26.7 ± 1.87 b	25.5 ± 1.97 b	22.9 ± 1.81 b
Cu (μg g^{-1})						
C. odorata	leaves	5.21 ± 0.82 b	8.50 ± 0.64 a	7.98 ± 0.89 ab	9.28 ± 1.60 ab	5.44 ± 0.52 b
	roots	8.55 ± 1.20 b	11.0 ± 1.03 ab	11.9 ± 0.84 ab	14.1 ± 0.82 a	14.4 ± 0.95 a
H. americanus	leaves	3.98 ± 0.28 b	5.06 ± 0.21 a	7.59 ± 1.59 ab	5.14 ± 0.17 a	5.08 ± 0.37 ab
	roots	6.42 ± 0.38 c	9.58 ± 0.47 b	14.1 ± 2.23 abc	11.9 ± 0.57 a	14.2 ± 1.14 a
T. chrysantha	leaves	6.13 ± 0.35 a	4.58 ± 0.25 bc	5.89 ± 0.51 ab	3.58 ± 0.25 c	3.55 ± 0.44 c
	roots	12.0 ± 1.13	11.0 ± 1.04	12.3 ± 0.54	14.5 ± 0.77	13.5 ± 0.82
Ni (μg g^{-1})						
C. odorata	leaves	3.41 ± 0.36 a	4.46 ± 0.23 a	3.75 ± 0.46 a	3.77 ± 0.30 a	1.33 ± 0.08 b
	roots	5.68 ± 0.70 ab	7.70 ± 0.76 a	5.88 ± 0.55 ab	4.21 ± 0.59 b	1.77 ± 0.12 c
H. americanus	leaves	3.97 ± 0.24 bc	5.49 ± 0.17 a	5.67 ± 0.98 abc	4.38 ± 0.08 b	3.41 ± 0.19 c
	roots	1.59 ± 0.15 c	5.00 ± 0.34 a	3.38 ± 0.20 b	1.10 ± 0.17 c	0.45 ± 0.06 d
T. chrysantha	leaves	3.29 ± 0.42 a	3.33 ± 0.40 a	2.68 ± 0.14 ab	1.47 ± 0.35 bc	0.63 ± 0.05 c
	roots	12.0 ± 0.74 a	11.3 ± 0.99 a	4.78 ± 0.56 b	3.31 ± 0.21 b	1.89 ± 0.21 c
Al (mg g^{-1})						
C. odorata	leaves	0.02 ± 0.01 c	0.09 ± 0.02 bc	0.12 ± 0.02 b	0.31 ± 0.05 a	0.41 ± 0.04 a
H. americanus	leaves	0.00 ± 0.0 d	0.00 ± 0.0 dc	0.06 ± 0.02 bc	0.11 ± 0.02 b	0.42 ± 0.08 a
T. chrysantha	leaves	0.01 ± 0.01 c	0.06 ± 0.02 c	0.18 ± 0.03 b	0.22 ± 0.03 b	0.63 ± 0.03 a

Data refer to means of 9 replicates ± SE and 8 replicates ± SE for *H. americanus* and *T. chrysantha* in the treatments with 0 and 2400 μM Al, as well as for roots of *C. odorata* in the 1200 μM Al treatment. Lower case letters depict significant differences among the treatments at $p < 0.05$. Al concentrations in roots of the studied tree species are given in Rehmus et al. (2014)

Aluminum concentrations in the leaves of all three tree species increased significantly with increasing Al exposure (Tab. 3.2).

3.4.3 Relationship between nutrient status and biomass production

Calcium and Mg concentrations in leaves correlated positively with shoot biomass (Tab. 3.3, Fig. 3.1). Furthermore, P concentrations in roots correlated significantly with root biomass in *T. chrysantha* (Fig. 3.2) but not in the other two species.

Concentrations of Al and P in roots correlated significantly (Fig. 3.3). In the roots of the individual tree species the correlation between Al and P was only significant for *C. odorata* ($r_s = 0.50$, $p < 0.001$, $n = 45$) and *T. chrysantha* ($r = 0.43$, $p = 0.004$, $n = 43$).

Table 3.3: Correlations between macronutrient concentrations in leaves and roots with shoot and root biomass, respectively, of all tree species together

	leaves			roots		
	r	p	n	r	p	n
N	-0.73[a]	< 0.001	130	-0.56[a]	< 0.001	122
P	-0.15[b]	0.08	131	0.24[b]	0.006	131
K	-0.74[b]	< 0.001	131	0.08[b]	0.36	130
Ca	0.33[b]	< 0.001	131	-0.08[b]	0.31	131
Mg	0.38[b]	< 0.001	130	0.18[b]	< 0.001	131
S	-0.16[a]	0.06	130	-0.02[b]	0.87	122

[a] Pearson product-moment correlation coefficient
[b] Spearman's rank correlation coefficient

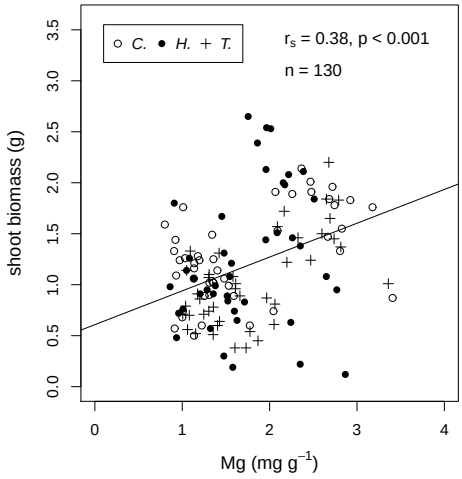

Figure 3.1: Relationship between Mg concentrations in leaves (mg g^{-1}) and shoot biomass (g) of all tree species in all Al treatments. r_s = Spearman's rank correlation coefficient. *C.* is *C. odorata*, *H.* is *H. americanus* and *T.* is *T. chrysantha*

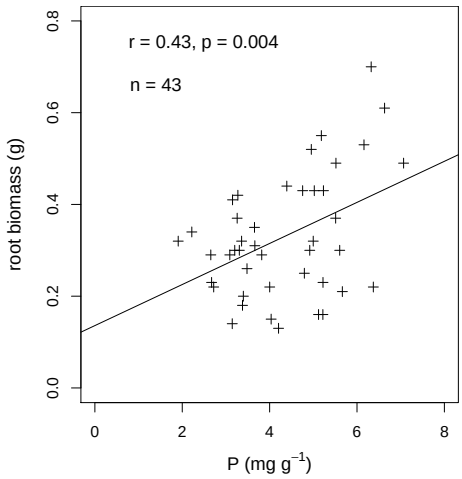

Figure 3.2: Relationship between P concentrations in roots (mg g^{-1}) and root biomass (g) of *T. chrysantha* in all Al treatments

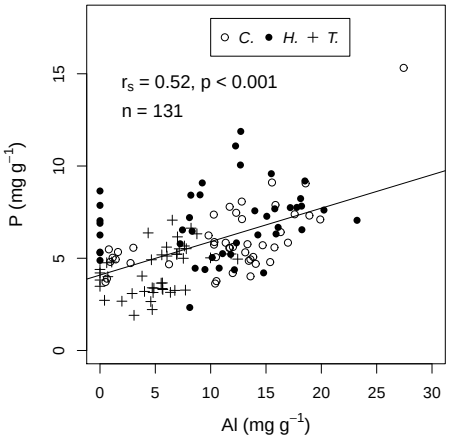

Figure 3.3: Relationship between Al and P concentrations (mg g^{-1}) in roots of all species and in all treatments. r_s = Spearman's rank correlation coefficient. *C.* is *C. odorata*, *H.* is *H. americanus* and *T.* is *T. chrysantha*

3.5 Discussion

3.5.1 Reduced shoot biomass

There is no published reference with respect to the specific nutrient demand of the investigated tree species. Hence, mineral concentrations in plant tissue can only be compared with general thresholds for optimum plant growth, usually derived for agricultural plants of the temperate zone, and to nutrient compositions of other woody plants and trees from tropical regions. We compared the mean nutrient concentrations in leaves and roots over all tree species and treatments to thresholds given by Amberger (1996) and Marschner (2012) for optimum growth of crop plants and to nutrient composition of tree leaves from a Brazilian Cerrado and from a tropical seasonal rain forest in southwest China (Tab. 3.4). Except for Mg concentrations, the nutrient concentrations were within ranges indicative for undisturbed nutrition.

Our finding that N concentrations increased while shoot biomass decreased with increasing Al concentration in nutrient solution clearly indicates that the Al effect on biomass production is not related with N supply. Decreasing biomass at consistently good N supply might have resulted in a concentration of N in the

Table 3.4: Range of selected mineral nutrient concentrations in plant tissue of crop plants required for optimal plant growth (Amberger 1996; Marschner 2012), in leaves of Brazilian Cerrado trees (Lilienfein et al. 2001), in tree leaves of a tropical seasonal rain forest in Southwest China (Shanmughavel et al. 2001), and mean ± SE and range of respective nutrients in plant tissue (leaves and roots) of all tree species and all Al treatments in our study

	Demand for optimal growth of crops		**leaves Cerrado trees**	**tree leaves southwest China**	**Concentrations present study**	
	Amberger (1996)	Marschner 2012	Lilienfein et al. (2001)	Shanmughavel et al. (2001)	mean ± SE	range
N (mg g^{-1})	5 - 50	10 - 50	9.7 – 28	7.4 – 10.5	35.0 ± 0.31	23.6 – 47.8
P (mg g^{-1})	1.5 - 8	3 - 5	0.48 – 1.6	0.8 – 1.0	4.27 ± 0.14	1.40 – 15.3
K (mg g^{-1})	5 – 50	20 – 50	1.4 – 5.9	7.5 – 10.6	30.4 ± 0.51	13.2 – 56.3
Ca (mg g^{-1})	<10 - 50	1 - >50	0.86 – 6.6	2.2 – 5.1	7.10 ± 0.27	1.81 – 21.0
Mg (mg g^{-1})	2 - 50	1.5 - 3.5	0.71 – 2.1	3.2 – 5.4	1.57 ± 0.04	0.51 – 3.55
S (mg g^{-1})	1 - 6	1 - 5	0.94 – 2.0	*	2.88 ± 0.06	1.57 – 7.26
Fe (μg g^{-1})	*	50 – 150	81 – 615	*	316 ± 28	20 – 3,856
Zn (μg g^{-1})	10 - 100	15 – 20	3.8 – 23	*	39.9 ± 1.55	12.2 – 155
Cu (μg g^{-1})	4 – 20	1 – 30	*	*	8.90 ± 0.28	1.78 – 31.0
Ni (μg g^{-1})	<1	1 – >10	*	*	4.05 ± 0.18	0.22 – 16.6

* no general range suggested/no data given

plants (Tab. 3.1). Aluminum is known to stimulate N uptake in plants like *Camellia sinensis* [L.] Kuntze which respond positively to increasing Al concentrations (Hajiboland et al. 2013a). Hajiboland et al. (2013a) attributed this observation to impaired protein synthesis by Al.

Concentrations of P in plant tissue were strongly affected by Al (Tab. 3.1). As the P concentrations in leaves of all tree species decreased in the Al treatments compared to the control, a deteriorated P supply in the leaves could have contributed to reduced shoot biomass production. Increasing P concentrations in leaves and roots of *H. americanus* and *T. chrysantha* in the highest Al treatments might result from a P concentration effect because of strongly reduced leaf biomass.

In our study, the K concentrations in the plant tissue increased while Ca and Mg concentrations decreased. This is inconsistent with other observations in the literature (Dogan et al. 2014). Thus, increasing Al concentrations seemed to inhibit Mg and Ca uptake by blocking specific channels (Rengel 1992), while K

might have been unaffected by Al and even benefited from reduced ion competition.

Magnesium has a crucial function in the photosynthesis of green plants, because Mg is the central ion of the chlorophyll molecule (Marschner 2012). The Mg concentrations in leaves and roots treated with 600 μM Al and more were close to and even below the threshold for nutrient deficiency (1.5 mg g^{-1}, Marschner 2012) (Tab.s 3.1 and 3.4). The closest positive correlation occurred between Mg concentrations in leaves and shoot biomass (Fig. 3.1). Similarly, the correlation between Ca concentrations and shoot biomass production was highly significant yet not as close (Tab. 3.3). Thus, Mg deficiency might cause reduced photosynthesis and inhibited biomass production. Indeed, the symptoms on the leaf surface of *C. odorata* and *H. americanus* (Rehmus et al. 2014) were similar to those described by Amberger (1996) for Mg deficiency. Speckled patches of chlorophyll defects developed over time to necrosis. An Al-induced reduction in chlorophyll concentration and consequently reduced photosynthesis in different plant species has been reported in several studies (e.g. Ali et al. 2008; Silva et al. 2012; Zhang et al. 2007). A stronger decrease of the Mg concentrations in roots than in leaves (Tab. 3.1) might reflect the attempt of the plant to supply the leaves with Mg rather than the roots to maintain the photosynthesis functioning. However, it should be considered that nutrient demand of crop plants is higher than that of trees. Sun and Payn (1999) found the photosynthesis rate of *Pinus radiata* D. Don to be reduced at Mg concentrations in shoots below 0.6 mg g^{-1}. Laing et al. (2000) observed a strongly decreased photosynthesis in *P. radiata* at Mg concentrations in needles of 0.2 – 0.25 mg g^{-1}. The mean concentrations of Mg in our study were 1.57±0.04 mg g^{-1}, which is higher than reported critical values of Sun and Payn (1999) and Laing et al. (2000). Thus, a suppressed Mg uptake might not be the only reason for negative effects on plant growth.

Concentrations of S were partly affected by increasing Al concentrations in nutrient solution. As even the lowest S concentrations were higher than thresholds suggested for optimum plant growth (Tab. 3.4), a considerable role of S in reduced shoot biomass production is unlikely.

Although micronutrients partly showed consistent responses to increased Al concentrations in nutrient solution (Tab. 3.2), their mean concentrations were

in the range of thresholds for optimal plant growth, suggesting that micronutrient deficiencies are unlikely. Also toxic effects of micro elements like Fe are not likely as mean Fe concentrations in leaves (Tab. 3.2) were clearly below the suggested critical value for Fe toxicity which is 500 $\mu g\ g^{-1}$ (Marschner 2012). High Fe concentrations in roots of *C. odorata* might be attributable to Fe binding or precipitation and detoxification on the root surface or in the roots, as translocation of Fe to the leaves was low (Tab. 3.2).

Energy-consuming defense mechanisms against Al toxicity like extrusion of Al-complexing organic acids might further weaken the plants (Cuenca et al. 1990; Ma et al. 2001). This observation is in line with our finding that TOC concentrations in the nutrient solution increased with increasing Al concentrations, probably attributable to organic acid exudation (Rehmus et al. 2014).

3.5.2 Stimulated root growth at 300 μM Al

Alleviation of latent Fe stress by exposure to 200 μM Al in nutrient solution and decrease of Fe concentrations in young leaves and roots as the driving mechanism for growth stimulation in tea plants (*Camellia sinensis* (L.) O. Kuntze) was suggested recently by Hajiboland et al. (2013b). In our study, exposure to 300 μM Al caused the inverse effect: Fe concentrations in roots of *C. odorata* increased significantly compared to the control. Alleviation of Fe toxicity by low Al concentrations can therefore be ruled out as mechanism supporting growth stimulation.

The P concentrations in roots treated with 300 μM Al were higher for *C. odorata* and *T. chrysantha* compared to the control (Tab. 3.1). This might indicate that improved P supply at low Al concentrations is the reason for stimulated root biomass production of *T. chrysantha*. In fact, we found a positive and significant correlation between root biomass and P concentrations in the root tissue of *T. chrysantha* (Fig. 3.2) supporting our assumption, that root biomass production is limited by P uptake. This effect would be in line with hormesis because of substrate amelioration according to Poschenrieder et al. (2013). Improved P supply by elevated Al concentrations below toxicity thresholds might even be more important in the P-poorer organic layer leachates.

The P translocation into the leaves seemed to be suppressed by Al as the P concentrations were significantly higher in roots than in the leaves at Al concentrations ≥300 μM. This suggests that Al-phosphates precipitated on the root surface or in the roots.

Correlations between P and Al concentrations in roots are highly significant for *C. odorata* and *H. americanus* but not for *T. chrysantha*. The relationship between P and Al concentrations is in line with findings by Jiang et al. (2009) that P can alleviate Al stress, probably by precipitation of Al-P compounds on the root surface and/or in the root tissue. Increased Al resistance has been reported to be attributable to detoxification by precipitation of Al phosphates in the roots (Zheng et al. 2005; Gaume et al. 2001). Alternatively, stimulated exudation of Al-chelating organic acids (Liao et al. 2006) and a shift from oxalate to citrate exudation, preventing the precipitation of Ca-oxalate in the root cytoplasm and xylem sap (Watanabe and Osaki 2001), has been reported to enhance Al tolerance and plant growth. However, Al-P precipitation in the roots and exudation of organic acids are mechanisms to counteract Al toxicity and do not explain an improved root biomass production compared to the Al free control. It has been shown that essential as well as non-essential metal ions, which can be even toxic to plants, can reduce biotic stress at low concentrations by counteracting pathogens and herbivorous damage, depending on the toxicity threshold of plant species and invaders (Poschenrieder et al. 2006, 2013). Thus, changes in P uptake and alleviation of biotic stress might be the reason for root growth stimulation at low Al concentrations.

3.6 Conclusions

i) Although the uptake of most crucial mineral nutrients, except for N, K, and the micronutrients, was impaired by specific Al concentrations, the element concentrations in plant tissue were mostly above tresholds for nutrient deficiency, except for Mg. Therefore, a disturbed Mg supply and consequently reduced photosynthesis is likely a major reason for low biomass production above 300 μM Al in hydroponic solution.

ii) The stimulated P uptake in the treatment with 300 μM Al seems to promote root biomass production of *T. chrysantha*. The role of Al-induced biotic stress alleviation in the studied native montane forest trees still needs to be elucidated.

3.7 Acknowledgments

We thank Karoline Klaus, Katharina Kitzinger, Jose Luis Peña Caivinagua, and Orly Mendoza Aguirre for support in set up of the experiment, Nature and Culture International in Loja, EC, for providing the study area and the research station, Ecuadorian authorities for the research permit, and the German Research Foundation (DFG) for funding (FOR 816, Wi 1601/8-2).

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4. Response of Al concentrations and speciation in soil solution to Ca amendment in a tropical montane forest in southern Ecuador

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4.1 Abstract

Soils of tropical montane forests frequently have low pH values and thus toxic Al might affect ecosystem functioning. However, toxicity of Al depends on Al speciation and concentrations of antagonistic ions like Ca^{2+} and Mg^{2+}. We analyzed organic layer leachate (LL) and mineral soil solutions (at the 0.15 m and 0.30 m soil depths, SS15 and SS30) from four replicate plots of each of untreated control and $CaCl_2$ amendment at a rate of 10 kg ha^{-1} yr^{-1} of Ca. We determined Al, Ca, Cl^-, dissolved organic carbon (DOC), F^-, K, Mg, Na, NH_4^+, NO_3^-, pH, PO_4^{3-}, and SO_4^{2-} concentrations and electrical conductivity (EC) to model Al speciation. Additionally, we determined free Al concentrations with the Donnan membrane technique (DMT). Modeled (here and in the following mean±SE: LL 0.56±0.21 %, SS15 0.53±0.25 %, SS30 0.87±0.32 % of total Al are cationic Al species) and DMT-measured free Al concentrations (LL 0.68±0.41 %, SS15 0.52±0.20 %, SS30 0.93±0.26 % of total Al are cationic Al species) showed good agreement. The degree of organic complexation of Al was very high (99.58±0.09 %). The resulting very low free Al concentrations are not likely to affect plant growth. The concentrations of potentially toxic Al^{3+} increased with soil depth (0.00±0.00 μM, 0.01±0.01 μM, and 0.25±0.06 μM in LL, SS15, and SS30 respectively), because of higher total Al and lower DOC concentrations in soil solutions. The Ca additions caused an increase of Al in LL, probably because Al^{3+} was exchanged against the added Ca^{2+} ions while pH remained constant. The total Ca:total Al molar ratios were close to 1 and sometimes < 1 which is usually interpreted as indication of the risk of Al toxicity. However, free ion molar ratios of Ca^{2+}:Al^{3+} were wide (374±69), because of a much higher degree of organo-complexation of Al than Ca. We conclude that dissolved organic matter concentrations detoxify Al in acidic tropical forest soils.

4.2 Introduction

Soils of tropical montane forests frequently have low pH values, which are known to increase plant available Al concentrations in soil solution. Consequently, Al toxicity was suggested to affect plant growth and ecosystem functioning of tropical

montane forests (Bruijnzeel and Veneklaas 1998; Hafkenscheid 2000; Leuschner et al. 2007). Our long-term study of all major ecosystem fluxes in a tropical montane forest in south Ecuador revealed acidification of the ecosystem by deposition of mineral acids and acidifying N deposition (Boy et al. 2008; Wilcke et al. 2013a,b). The mineral acid deposition originated from the anthropogenically increased vegetation fires in the Amazon basin (Boy et al. 2008). Although episodic Sahara dust deposition partly counteracted this acidification, there was a significantly decreasing trend in the pH of organic layer leachates (LL) between 1999 and 2009 (own unpublished results). We suggest that the continuous acidification can be attributed to enhanced nitrification because of the strongly increasing NH_4^+ deposition between 1999 and 2009 (Wilcke et al. 2013b) which was associated with increasing nitrate leaching (Wilcke et al. 2013a) in addition to the mineral acid deposition. Thus, the potential risk of Al toxicity might even increase in the near future.

Up to now, Al toxicity has been mainly studied for crop plants and trees of economic relevance (Kinraide 2003; Schaedle et al. 1989), while data about Al sensitivity of native tropical tree species are scarce. Cuenca et al. (1990) described adaption mechanisms of tropical trees to Al stress in acidic soils and differences in Al accumulating trees compared to Al non-accumulating trees. Watanabe et al. (1998) investigated the effect of Al on tropical Al accumulating species and found an increase in tree growth at concentrations of 500 μM Al in the growth solution. A reduced aboveground biomass production by 10 % at Al concentrations of 126 – 376 μM (EC10) and simultaneous stimulation of root biomass production at 300 μM Al in seedlings of tree species which are common in the south Ecuadorian montane forests was reported by Rehmus et al. (2014a).

The total Al concentration in soil solution often failed to describe Al toxicity because of the differential toxicity of the various Al species (Kinraide 1991, 1997). The free Al^{3+} ion, the mononuclear $Al(OH)^{2+}$ and $Al(OH)_2^+$ ions and polynuclear Al species (e.g., $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$ which is in the literature referred to as Al_{13}) are phytotoxic. Fluoride complexes (AlF^{2+} and AlF_2^+) are considered to be toxic as well, while sulfate complexes and organically complexed Al are supposed to be nontoxic (Alva et al. 1986; Buyukkeskin and Akinci 2011; Cronan and Grigal 1995; Kinraide 1997). The strongly toxic polynuclear Al species (e.g.,

Al_{13}, Kinraide 1991) can form in solution if fast changes of pH or Al concentrations occur and Al concentrations are $> 10\ \mu M$ (Furrer et al. 1992). In the thick organic layers of our study site in the Ecuadorian tropical montane forest, dissolved Al concentrations are comparatively low (Wullaert et al. 2013). Furthermore, geochemical speciation modeling suggested that Al in the LL is almost entirely organically complexed (57 – 100 %, mean 99 %) because of high dissolved organic carbon (DOC) concentrations (Wullaert et al. 2013). The Al concentrations in mineral soil solutions are supposed to be higher than in LL because of the higher concentrations of Al oxides in the mineral soil. The Al speciation is supposed to turn increasingly inorganic because of lower DOC in soil solutions. Alleoni et al. (2010) and Vieira et al. (2009) reported that 30 – 99 % of the Al in soil solution is organically complexed in subtropical and tropical agricultural soils. However, the thermodynamic speciation modeling of Al used by Alleoni et al. (2010), Vieira et al. (2009), and Wullaert et al. (2013) is based on a number of uncertain assumptions, particularly with respect to the Al-complexing properties of dissolved organic matter (DOM). Therefore, there is an additional need to directly measure free Al ion concentrations with the Donnan membrane technique (DMT; Temminghoff et al. 2000) to support modeling results.

Aluminium toxicity furthermore depends on the presence of competing cations such as Ca and Mg in soil solution. High Ca and Mg concentrations are known to alleviate Al toxicity (Cronan and Grigal 1995). Consequently, the episodic Ca and Mg inputs originating from the Sahara into the tropical montane forests on the rim of the Amazon basin observed by Boy and Wilcke (2008) would reduce Al toxicity. To assess the effect of low-level Ca addition on the ecosystem, in 2008 a Ca amendment experiment with annual application of Ca as pH neutral $CaCl_2$ was started in the frame of the Nutrient Manipulation Experiment (NUMEX, Wullaert et al. 2010, 2013; Homeier et al. 2012). Our overall objective was to determine Al speciation by DMT measurements and thermodynamic modeling and the response of Al concentrations and speciation to Ca amendment in the organic layers and mineral soil solutions.

We hypothesize that

i) free Al concentrations as determined with DMT match the modeled values confirming thermodynamic speciation modeling as a valuable tool to assess Al speciation,

ii) total and potentially toxic inorganic Al is in all soil depths in a range which is nontoxic for tropical tree species,

iii) free Al concentrations are lowest in the LL and increase in the mineral soil solutions, attributable to lower DOC concentrations and higher total Al concentrations, and

iv) Ca amendments increase Ca:Al molar ratios in the soil solution and therefore decrease the threat of Al toxicity.

While Wullaert et al. (2013) found no effect of Ca addition on Al speciation and Ca:Al ratios in the LL after 3 Ca applications, our dataset includes two further application and mineral soil solutions increasing the likelihood to identify effects of Ca additions.

4.3 Materials & Methods

4.3.1 Study site and sampling procedures

The study site is located on the eastern slope of the eastern Andean cordillera (Cordillera Real) in southern Ecuador between the cities of Loja and Zamora (4°00' S and 79°05' W) at an altitude ca. 2020 – 2120 m a.s.l. The study site has an average slope of 51 % (range: 25 – 84 %). The mean annual rainfall between 1999 and 2009 measured at 2 – 4 gauging stations with five rain collectors at each station was 2400±180 (SD) mm. Rainfall has a unimodal distribution with a maximum between April and September and without a pronounced dry season (Fleischbein et al. 2005, 2006). The vegetation is classified as lower montane forest (Bruijnzeel and Hamilton 2000). The dominating soils are Stagnic Cambisols (Hyperdystric, Chromic) (IUSS Working Group WRB 2007) developed from Palaeozoic phyllites, quartzites and metasandstones.

We studied the untreated control and the Ca addition plots of NUMEX. Each treatment was fourfold replicated. The unfertilized control plots were located upslope to avoid nutrient leaching from fertilized to control plots. The location of each plot was selected in a way that the vegetation was representative of the area and similar on all plots. Each plot was 400 m^2 (20 m x 20 m) large and the distance between the plots was at least 10 m. The Ca-addition plots received 10 kg Ca ha^{-1} yr^{-1}, split in two applications per year. This is equivalent to ca. 2.5 times the mean annual Ca deposition with rainfall of 4.0 kg ha^{-1} yr^{-1} between 1999 and 2009. Calcium additions started in January 2008, followed by further applications in August 2008, February 2009, July 2009, and February 2010. Calcium was applied manually in solid phase as a fine-grained $CaCl_2 \cdot 2H_2O$ salt in pro analysi quality.

Three zero-tension lysimeters per plot were used to collect leachate from the organic layer. The lysimeters were made of plastic boxes with a 0.15 m x 0.15 m collection area and covered with a polyethylene net with a mesh width of 0.5 mm. The lysimeters were introduced from the wall of soil pit to minimize disturbance of the organic layer. The lysimeters were pushed ca. 0.50 m into the soil so that they were entirely covered by the organic layer. Soil solution at the 0.15 m and 0.30 m (SS15 and SS30, respectively) mineral soil depths was collected using suction lysimeters (ceramic suction cups with 1 μm pore size). Soil solution samples were collected fortnightly between September 2009 and October 2010. To guarantee Al saturation of the ceramic cups to prevent Al sorption to the ceramic material during the sampling period, soil solution was extracted from August 2007 on allowing for equilibration of the cups during two years.

4.3.2 Chemical analyses

After collection in the field, LL and mineral soil solution samples were transported to our field laboratory where first electric conductivity (ProfiLine Cond 3110, WTW GmbH, Weilheim, Germany) and then pH (Sentix HWS, WTW GmbH, Weilheim, Germany) was immediately measured in an unfiltered aliquot of each sample within < 24 h. Another aliquot was filtered (ashless folded paper filters with pore size 4 – 7 μm, Type 389; Munktell & Filtrak GmbH, Bärenstein,

Germany) and frozen until transport to Germany or Switzerland for further analyses. During transport, samples only partly melted so that their temperatures hardly exceeded 0°C.

Concentrations of Al were determined by inductively-coupled plasma mass spectrometry (7700x, Agilent Technologies, Santa Clara, CA). Concentrations of Ca, K, Mg, and Na were determined with flame atomic absorption spectrometry (Varian AA240FS, Thermo Fisher, Darmstadt, Germany or ZEEnit 700P, Jena Analytik, Jena, Germany). Samples were furthermore analyzed for concentrations of total dissolved Cl^-, NH_4^+, NO_3^-, and PO_4^{3-} using continuous flow analysis (CFA, AutoAnalyzer 3, Seal Analytical GmbH, Norderstedt, Germany). Sulfate was determined by ion chromatography (Dionex ICS-900, Thermo Scientific, Waltham, MA, USA) after filtration (0.45 μm pore size). Fluoride concentrations were determined with an ion-sensitive electrode (WTW Inolab pH/Ion 735 with a WTW F800 electrode, WTW, Weilheim, Germany) after addition of TISAB III (Fluka Analytical) by standard addition. Dissolved organic carbon (DOC) concentrations in LL were measured with a high temperature TOC analyzer (varioTOC cube, Elementar Analysensysteme, Hanau, Germany).

4.3.3 Donnan membrane technique

To validate the results of the chemical speciation modeling, we determined the free Al concentrations in soil solutions using the Donnan membrane technique (DMT; Sigg et al. 2006; Temminghoff et al. 2000; Weng et al. 2004). Prior to application, all material used for the DMT was carefully cleaned in an acid bath and rinsed three times with deionized water (> 18.2 MΩ). Afterwards, 600 – 2000 mL of the sample solution (donor solution) were filled in a plastic beaker and placed on a magnetic stirrer. A Donnan membrane field cell which consists of an acryl frame with two negatively charged membranes (BDH Prolabo, No. 55165 2U) which enclose an acceptor solution (Weng et al. 2004) was sunk into the sample in a way that membranes on both sides were accessible for the donor solution. The beaker was sealed with parafilm and covered with a black plastic bucket to protect it from light. The ionic strength of the acceptor solution was adjusted according to the mean ionic strength in the sample solution using $CaCl_2$ (Trace select, Sigma

Aldrich, Buchs, Switzerland). After four days of equilibration (Weng et al. 2004), the cells were recovered and the acceptor solution was sampled and directly acidified. The donor solution was characterized in the same way as the soil solution samples. In the acceptor solutions, Al and Na concentrations were determined. Aluminum concentrations were corrected for ionic imbalances between donor and acceptor solutions using Na concentrations (Temminghoff et al. 2000). Because only positively charged ions diffuse through the negatively charged membranes, the corrected Al concentrations in the acceptor solutions indicate concentrations of 'free' cationic Al species (Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^+$, AlF^{2+} etc.), which are considered as phytotoxic. Most nontoxic, neutral and negatively charged species e.g., Al complexed by DOM, are not transferred to the acceptor solution (Temminghoff et al. 2000; Weng et al. 2002). The DMT experiment was realized shortly after sampling directly in our field laboratory in Ecuador. Overall, we conducted 12 DMT measurements (at least in triplicate per solution type, LL, n = 4; SS15, n = 3; SS30, n = 5).

4.3.4 Speciation modeling and statistics

The speciation of Al in LL was modeled with measured data for solution composition (Al, Ca, Cl^-, DOC, EC, F^-, K, Mg, Na, NH_4^+, NO_3^-, pH, PO_4^{3-}, SO_4^{2-}, Tab. 4.1) using the geochemical code Visual MINTEQ (VMINTEQ, Version 3.0 beta, J.P. Gustafsson). Within VMINTEQ, the NICA-Donnan model was used to assess complexation of Al with humic substances. Details about the NICA-Donnan model are given in Kinniburgh et al. (1996). NICA-Donnan properties of metal complexation by organic acids were taken from the literature (Milne et al. 2003). An active DOM/DOC ratio of 2 was assumed and the dissolved organic acids were adjusted to be 100 % fulvic acids. Toxic polynuclear Al species (e.g., Al_{13}, Kinraide 1991) are not considered in the model. However, Al_{13} was not supposed to exist in solution in the forest soil because no strong changes in Al concentrations or pH occurred and total dissolved Al concentrations were generally low (Tab. 4.2).

Aluminum speciation was modeled for 308 samples of LL, SS15, and SS30. The F^- concentrations were determined for a subset of 176 samples from all

Table 4.1: Summary of pH, electric conductivity (EC), ionic strength, Ca, Cl^-, DON, K, Mg, Na, NH_4-N, NO_3-N, o-PO_4-P, TN, TP, and DOC concentrations used for the speciation modeling in organic layer leachate (LL) and mineral soil solution in the 0.15 m (SS15) and 0.30 m (SS30) soil depths in control and Ca-amended NUMEX plots between September 2009 and October 2010

Sample type		pH	EC μS cm^{-1}	ionic strength mmoleq L^{-1}	Ca	Cl	DON	K	Mg	Na	NH4-N μM	NO3-N	o-PO4-P	TN	TP	DOC
Control																
	median	4.0	53.8	0.70	3.98	25.9	52.2	12.3	6.13	8.68	58.5	1.57	0.65	120	0.81	3.61
LL	mean	4.0	60.7	0.78	9.13	28.9	76.9	21.1	11.6	9.10	64.8	2.82	2.34	145	8.39	3.96
(n = 61)	min.	3.5	26.1	0.34	0.09	9.20	0.57	1.34	1.06	3.32	34.0	0.00	0.00	64.3	0.00	2.01
	max.	5.1	141	1.51	46.3	94.0	1460	131	68.7	20.2	128	12.7	49.4	1570	88.9	8.37
	median	4.4	21.5	0.28	1.42	7.59	20.6	4.01	1.18	3.85	3.07	1.14	0.16	24.2	0.29	1.48
SS15	mean	4.3	24.7	0.33	4.67	10.8	21.2	4.32	1.97	4.74	8.24	2.32	0.18	31.8	4.42	1.62
(n = 51)	min.	3.7	5.80	0.08	0.09	0.00	0.43	0.23	0.04	0.00	0.36	0.00	0.00	2.64	0.00	0.26
	max.	6.1	55.8	0.73	25.2	66.5	57.8	13.9	10.7	20.1	105	23.1	0.97	154	76.2	3.25
	median	4.8	14.1	0.18	1.16	8.72	12.7	3.97	1.00	3.68	2.57	1.07	0.06	16.1	0.23	0.86
SS30	mean	4.5	13.8	0.18	4.86	9.68	12.8	3.62	1.62	4.71	4.23	1.53	0.12	18.6	2.58	0.85
(n = 45)	min.	3.9	4.30	0.06	0.09	0.00	2.57	0.00	0.04	0.05	0.00	0.00	0.00	3.78	0.00	0.23
	max.	5.6	24.6	0.32	27.2	29.6	31.0	7.29	8.64	20.2	34.6	10.3	0.90	61.6	17.8	2.03
Ca amended																
	median	4.0	74.6	0.95	3.96	29.2	53.5	13.7	5.71	7.86	58.7	2.50	0.76	117	0.89	4.10
LL	mean	3.9	73.2	0.96	7.77	37.6	57.7	18.2	8.94	9.64	60.2	4.77	1.54	123	6.43	4.07
(n = 60)	min.	3.5	37.9	0.49	0.50	12.4	10.8	1.45	0.75	3.78	32.8	0.00	0.00	54.2	0.14	1.01
	max.	5.1	126	1.83	46.8	288	255	82.6	54.6	40.4	93.6	34.3	33.4	312	107	6.81
	median	4.2	34.3	0.45	1.88	24.5	23.6	3.52	2.29	3.28	3.78	1.50	0.16	30.3	0.36	2.01
SS15	mean	4.2	34.0	0.44	6.21	25.4	22.0	3.90	3.22	4.50	7.56	5.29	0.20	34.9	3.10	1.93
(n = 45)	min.	3.7	12.1	0.16	0.09	5.19	2.86	0.28	0.04	0.00	0.43	0.00	0.00	9.64	0.00	0.61
	max.	5.7	55.9	0.73	51.6	59.6	41.8	10.4	14.7	32.0	80.8	100	1.07	128	33.6	3.30
	median	4.5	20.0	0.26	1.07	29.5	14.6	3.30	2.06	3.58	2.57	1.64	0.10	20.7	0.26	0.88
SS30	mean	4.4	19.9	0.26	4.67	29.7	19.3	3.97	2.37	3.97	4.10	7.62	0.09	31.0	4.93	0.89
(n = 45)	min.	3.6	8.40	0.11	0.04	14.2	4.21	0.00	0.04	0.62	0.07	0.14	0.00	9.99	0.03	0.52
	max.	6.4	58.4	0.76	34.6	62.9	211	11.0	11.4	9.52	39.1	83.2	0.32	217	43.2	1.49

three solution types and were in all cases below limit of quantification (0.81 μM) and in 89 % of the cases even below the limit of detection (0.26 μM). Thus, for the calculation we set F^- concentrations of all samples to half of the detection limit (0.13 μM). Sulfate concentrations were only available for 31 samples. For all samples without measured SO_4^{2-} concentrations, values in the model were set to 0.61 mg L^{-1} (i.e. the mean sulfate concentration of the measured samples). Ionic strength was estimated from electrical conductivity (Tab. 4.1) according to Griffin and Jurinak (1973). A Bonferroni-corrected Kruskal-Wallis test was used to test for significant differences between Ca amended and control plots and among LL and soil solutions from the different soil depths (Tab. 4.3 and 4.4). Corresponding leached ions in solutions of Ca amended plots were tested with Pearson Product Moment Correlations.

4.4 Results

The modeled free Al concentrations show good agreement with the free Al concentrations determined with the DMT approach (Fig. 4.1). According to the model results, the majority of the Al species in soil solutions are electrostatically bound to organic matter, while a smaller part of Al is specifically bound to functional groups of organic acids in soil solution (Tab. 4.2). In total, almost 100 % of Al in LL is bound to dissolved organic matter. The small portion of inorganic Al is dominated by Al^{3+} followed by AlF^{2+} and to a lesser extent by $Al(OH)^{2+}$, while other inorganic species showed only negligible contributions.

Total Al concentrations in all soil solutions were always far below the lowest EC10 level of 126 μM determined for seedlings of three native tree species by Rehmus et al. (2014a) (Tab. 4.2). The Ca:Al and Mg:Al molar ratios in all solutions were close to 1 and 73 % of the Ca:Al and 82 % of the Mg:Al molar ratios were below 1 (Tab. 4.2). However, because most Al was organically bound, the Ca^{2+}:Al^{3+} and Mg^{2+}:Al^{3+} molar ratios were far wider (on average ca. 370) and only around 8.5 % of the samples showed ratios < 1. Total Al concentrations increased significantly in the mineral soil solutions relative to LL (Fig. 4.2). This increase in concentrations with increasing soil depth was particularly pronounced for the potentially toxic free Al^{3+} species (Fig. 4.2, Tab. 4.3). There was a signif-

Table 4.2: Summary of total Al concentrations, Al speciation and species fraction in % of total Al concentrations, and Ca:Al, Ca^{2+}:Al^{3+}, Mg:Al, and Mg^{2+}:Al^{3+} molar ratios in organic layer leachate (LL) and mineral soil solution in the 0.15 m (SS15) and 0.30 m (SS30) soil depth in control and Ca-amended NUMEX plots between September 2009 and October 2010

Sample type		Al total	Al^{3+}		other anorganic Al		electrostatically bound to DOM		Specifically bound to DOM		Ca:Al	Ca^{2+}:Al^{3+}	Mg:Al	Mg^{2+}:Al^{3+}
		μM		%	μM	%	μM	%	μM	%	molar ratio			
All samples (n = 308)	median	14.3	0.08	0.00	0.00	0.00	13.8	96.2	0.49	3.66	0.27	102	0.21	70.2
	mean	16.0	0.08	0.30	0.02	0.12	15.3	94.5	0.66	5.06	0.91	374	0.70	373
	min.	0.12	0.00	0.00	0.00	0.00	0.08	5.63	0.02	1.93	0.00	0.03	0.00	0.00
	max.	54.7	3.94	11.1	0.73	4.33	51.7	98.1	3.02	93.3	40.5	17542	17.5	19352
Control														
LL (n = 61)	median	9.43	0.00	0.00	0.00	0.00	8.99	95.4	0.44	4.58	0.62	226	0.94	272
	mean	10.8	0.00	0.01	0.00	0.01	10.4	94.6	0.52	5.35	1.01	407	1.31	613
	min.	1.15	0.00	0.00	0.00	0.00	1.15	85.1	0.12	2.72	0.01	1.69	0.06	30.2
	max.	27.7	0.01	0.09	0.01	0.10	26.3	97.3	1.72	14.7	4.71	2115	5.54	2785
SS15 (n = 51)	median	18.8	0.00	0.00	0.00	0.00	17.9	97.4	0.47	2.58	0.14	50.1	0.08	33.3
	mean	18.9	0.01	0.02	0.00	0.01	18.3	96.4	0.56	3.61	0.54	401	0.22	129
	min.	0.49	0.00	0.00	0.00	0.00	0.46	71.0	0.03	2.10	0.00	0.65	0.00	0.31
	max.	48.6	0.05	0.14	0.02	0.17	47.4	97.9	1.83	28.7	9.66	6245	3.06	1682
SS30 (n = 45)	median	10.2	0.00	0.01	0.00	0.01	9.54	95.3	0.52	4.47	0.23	89.8	0.09	33.4
	mean	14.6	0.11	0.36	0.03	0.17	13.7	93.2	0.75	6.25	1.68	419	0.45	135
	min.	0.48	0.00	0.00	0.00	0.00	0.23	48.1	0.03	2.11	0.00	0.07	0.00	0.10
	max.	54.7	1.96	5.08	0.32	2.11	51.7	97.9	3.02	48.9	40.5	4726	8.02	1468
Ca amended														
LL (n = 60)	median	14.7	0.00	0.00	0.00	0.00	14.3	96.7	0.48	3.28	0.45	172	0.40	168
	mean	14.2	0.00	0.01	0.00	0.01	13.6	95.7	0.50	4.26	0.68	374	1.04	622
	min.	1.75	0.00	0.00	0.00	0.00	1.03	88.3	0.12	2.25	0.03	12.9	0.04	8.04
	max.	31.2	0.04	0.39	0.03	0.39	30.2	97.7	1.90	11.7	5.06	2266	9.06	4659
SS15 (n = 45)	median	22.4	0.00	0.01	0.00	0.01	22.0	97.3	0.53	2.64	0.11	30.6	0.13	27.7
	mean	20.9	0.02	0.10	0.01	0.07	20.3	94.7	0.67	5.15	1.11	463	0.34	137
	min.	1.35	0.00	0.00	0.00	0.00	0.08	5.63	0.19	1.93	0.00	0.08	0.00	0.00
	max.	44.2	0.48	2.17	0.12	1.02	43.2	98.1	1.99	93.3	38.4	17542	8.12	3690
SS30 (n = 45)	median	20.2	0.03	0.19	0.03	0.14	18.5	93.4	1.04	4.72	0.07	21.3	0.09	4.83
	mean	18.7	0.38	1.54	0.11	0.55	17.1	91.8	1.09	6.07	0.50	166	0.57	468
	min.	0.12	0.00	0.00	0.00	0.00	0.10	74.6	0.02	2.09	0.00	0.03	0.00	0.00
	max.	39.4	3.94	11.1	0.73	4.33	36.1	97.7	2.94	20.1	4.52	5005	17.49	19352

Table 4.3: Differences of the mean ranks of the compared groups in concentrations of total Al, Al^{3+}, sum of inorganic Al, Al specifically bound to DOM, Al electrostatically bound to DOM, total Ca, Ca^{2+}, and Ca:Al and Ca^{2+}:Al^{3+} molar ratios, Cl^{-}, ionic strength, total Mg, Mg^{2+}, Mg:Al and Mg^{2+}:Al^{3+} molar ratios, pH, and DOC among organic layer leachate (LL) and mineral soil solution in the 0.15 m (SS15) and 0.30 m (SS30) soil depth

Parameter	Comparison	Difference		Parameter	Comparison	Difference	
		Control	Ca amended			Control	Ca amended
Al total	LL - SS15	-21.9*	-32.2***	Cl^{-}	LL - SS15	63.0***	20.3
	LL - SS30	-7.17	-21.8*		LL - SS30	61.0***	2.74
	SS15-SS30	14.8	10.4		SS15-SS30	-1.96	-17.6
Al^{3+}	LL - SS15	-13.6	-24.5**	ionic strength	LL - SS15	59.7***	54.7***
	LL - SS30	-23.6*	-49.5***		LL - SS30	87.9***	87.9***
	SS15-SS30	-10.0	-25.0**		SS15-SS30	28.3***	33.2***
Al inorganic	LL - SS15	-15.4	-26.8***	Mg total	LL - SS15	59.4***	31.3***
	LL - SS30	-30.4**	-56.7***		LL - SS30	66.3***	41.3***
	SS15-SS30	-14.9	-29.9***		SS15-SS30	6.96	10.0
Al specifically	LL - SS15	1.97	-14.2	Mg^{2+}	LL - SS15	23.2*	-1.78
bound to DOM	LL - SS30	-2.12	-36.9***		LL - SS30	14.6	-18.3
	SS15-SS30	-4.10	-22.7*		SS15-SS30	-8.56	-16.6
Al electrostatically	LL - SS15	-21.7*	-32.2***	Mg:Al	LL - SS15	60.9***	40.1***
bound to DOM	LL - SS30	-6.07	-17.7	molar ratio	LL - SS30	58.6***	42.6***
	SS15-SS30	15.7	14.5		SS15-SS30	-2.29	2.53
Ca total	LL - SS15	38.0***	30.8***	Mg^{2+}:Al^{3+}	LL - SS15	53.1***	40.9***
	LL - SS30	37.1***	41.5***	molar ratio	LL - SS30	56.1***	59.1***
	SS15-SS30	-0.84	10.7		SS15-SS30	3.03	18.2*
Ca^{2+}	LL - SS15	8.85	-1.48	pH	LL - SS15	-32.1***	-25.5**
	LL - SS30	-6.37	-23.9*		LL - SS30	-58.2***	-47.0***
	SS15-SS30	-15.2	-22.4*		SS15-SS30	-26.1**	-21.5*
Ca:Al	LL - SS15	40.7***	36.3***	DOC	LL - SS15	57.8***	51.4***
molar ratio	LL - SS30	31.2***	38.6***		LL - SS30	90.2***	88.4***
	SS15-SS30	-9.55	2.29		SS15-SS30	32.4***	37.0***
Ca^{2+}:Al^{3+}	LL - SS15	37.1***	41.7***				
molar ratio	LL - SS30	31.6***	55.2***				
	SS15-SS30	-5.53	13.5				

Asterisks highlight the level of significance according to Kruskal-Wallis test with Bonferroni correction:
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

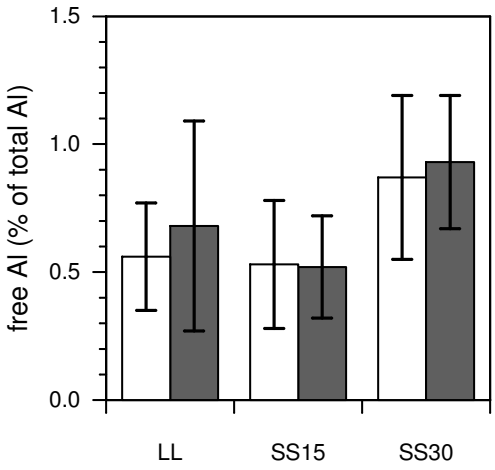

Figure 4.1: Comparison of free Al^{3+} concentrations (in % of total Al) as modeled with Vminteq (white bars) and analyzed with the DMT (grey bars) approach in organic layer leachate (LL) and soil solution in the 0.15 m (SS15) and 0.30 m (SS30) soil depth. Bar height indicates mean values while error bars indicate standard errors (LL, n = 4; SS15, n = 3; SS30, n = 5)

icant decrease of Ca, ionic strength, Mg, and DOC in SS15 and SS30 relative to LL, while pH increased. Because of higher Al and lower Ca and Mg concentrations in the mineral soil solutions, the molar ratios of Ca:Al, Mg:Al, Ca^{2+}:Al^{3+}, and Mg^{2+}:Al^{3+} narrowed with depth. The only difference in chemical properties between SS15 and SS30 referred to ionic strength, pH, and DOC concentrations.

The Ca amendment to the Ca plots increased total Al concentrations in LL. Additionally, electrostatically bound Al increased together with ionic strength in the LL and Mg:Al and Mg^{2+}:Al^{3+} molar ratios became narrower (Tab. 4.4). In SS15, inorganic Al was higher in the control, while ionic strength and Mg concentrations increased in the Ca amendment. The strongest effect occurred in Cl^- concentrations, which increased in the Ca amendment plots compared to the control (Tab. 4.3 and 4.4). In SS30, there was a number of significant differences between Ca-amended and control plots reaching from Al concentrations and speciation to ionic strength and Mg concentrations (Tab. 4.4). During the observation period from September 2009 to October 2010, concentrations of Cl^- correlated in the fortnightly samples with those of K^+ in LL ($p < 0.001$, $r = 0.43$) and SS15 ($p = 0.016$, $r = 0.36$), and with those of Na^+ in SS30 ($p = 0.020$, $r = 0.36$).

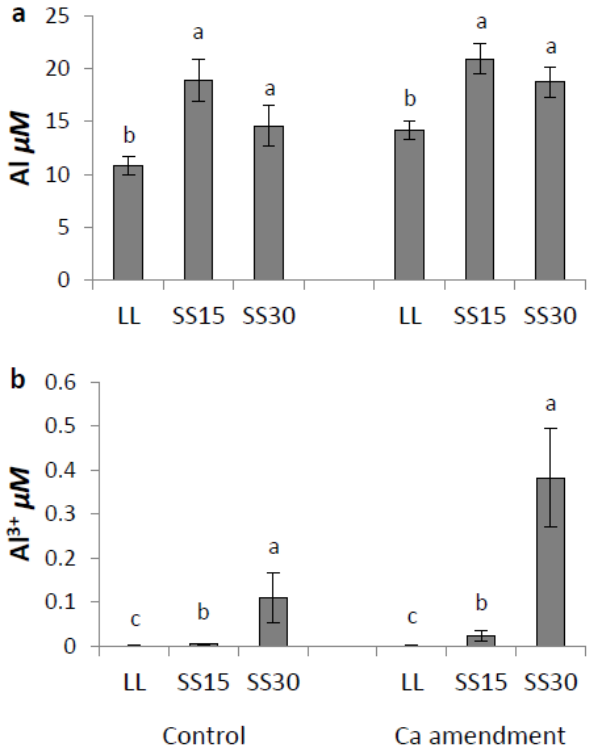

Figure 4.2: Total Al (a) and Al^{3+} (b) concentrations in organic layer leachate (LL) and mineral soil solution in the 0.15 m (SS15) and 0.30 m (SS30) soil depths in the Ca-amended and control plots of the Nutrient Manipulation Experiment (NUMEX) between September 2009 and October 2010. Bar height indicates mean values while error bars show standard errors. Lower case letters indicate significant differences ($p < 0.05$) between depths

The differences in Al speciation among the three considered soil depths were more pronounced in the Ca-amended plots than in the untreated control plots (Tab. 4.3).

Table 4.4: Differences of the mean ranks of the compared groups in concentrations of total Al, Al^{3+}, inorganic Al, Al specifically bound to DOM (Al spec. DOM), Al electrostatically bound to DOM (Al electrostat. DOM), total Ca, Ca^{2+}, Ca:Al and Ca^{2+}:Al^{3+} molar ratios, Cl^{-}, ionic strength, total Mg, Mg^{2+}, Mg:Al and Mg^{2+}:Al^{3+} molar ratios, pH, and DOC in organic layer leachate (LL) and mineral soil solution in the 0.15 m (SS15) and 0.30 m (SS30) soil depth between the control and the Ca-amended NUMEX plots between September 2009 and October 2010

Parameter	Difference		
	LL	SS15	SS30
Al total	16.9**	8.30	12.5*
Al^{3+}	5.55	11.0	17.4**
Al inorganic	4.76	11.5*	18.9***
Al spec. DOM	7.17	10.1	13.7*
Al electrostat. DOM	16.4**	8.14	11.9*
Ca total	0.12	3.33	-3.33
Ca^{2+}	1.79	6.46	10.2
Ca:Al	-12.3	-4.25	-9.49
Ca^{2+}:Al^{3+}	-9.16	-7.68	-16.5**
Cl^{-}	11.8	30.3***	41.0***
ionic strength	22.7***	18.7***	21.4***
Mg total	-8.99	14.6**	11.0*
Mg^{2+}	-2.74	11.1	16.1**
Mg:Al	-17.6**	5.63	0.11
Mg^{2+}:Al^{3+}	-14.2*	-2.95	-12.0*
pH	-8.18	-9.22	-9.69
DOC	7.79	10.1	2.60

Asterisks highlight the level of significance according to Kruskal-Wallis test with Bonferroni correction: *p < 0.05, **p < 0.01; ***p < 0.001

4.5 Discussion

The fact that the sum of the modeled concentrations of cationic Al species (Al^{3+}, $AlCl_2^+$, AlF^{2+}, AlF_2^+, $AlHPO_4^+$, $Al(OH)^{2+}$, $Al(OH)_2^+$, $Al_2(OH)_2^{4+}$, $Al_3(OH)_4^{5+}$,

$Al_2PO_4^{3+}$, $AlSO_4^+$) matched the experimentally determined free Al concentrations well (Fig. 4.1), indicates that thermodynamic speciation modeling of Al in soil solutions can be reliably used to predict Al speciation.

The effect levels of Al concentrations in the hydroponic experiment of Rehmus et al. (2014a) with seedlings of three native tree species of 126 – 376 μM (EC10) for aboveground biomass reduction are distinctly above the maximum level of total dissolved Al in soil solutions from our study site (Tab. 4.2), which renders Al toxicity for the investigated native trees unlikely. The latter becomes even more evident when it is considered that the dominating organically complexed Al is supposed to be not toxic. Consequently, an even smaller part of the total Al concentrations far below the 10 % effect level is potentially toxic (Fig. 4.2). Furthermore, Wheeler et al. (1992) reported a yield reduction of 50 % for Al-sensitive plants already at Al^{3+} concentrations < 1 μM in growth solution, while the most tolerant plants they investigated only showed a 50 % yield reduction at > 30 μM Al^{3+}. On our study site, Al^{3+} concentrations in the LL were always very low (≤ 0.04 μM), because of high complexation (99.97 % of total Al) by DOC (electrostatic and specific binding to functional groups of DOM). This high degree of organo-complexation can be explained by the comparatively low content of Al-bearing minerals (e.g., clay minerals, gibbsite) and high concentrations of organic acids in the organic soil layers. As a result, in the organic layer Al toxicity is unlikely even for the most sensitive plants. This is especially important because the organic layer in the whole NUMEX area is 0.15 m – 0.60 m (mean 0.30 m) thick and the place of germination and initial growth of all tree species. Furthermore, the organic layer is the main rooting zone for mature forest trees (Soethe et al. 2006; Wullaert et al. 2013).

The Al speciation changed between organic and mineral soil layers. While in organic layers, the low Al-containing mineral content causes a lower total Al concentration in LL, the total Al concentrations in solution increased in the SS compared to the LL (Tab. 4.3). In the organic layers, high DOC concentrations caused strong complexation, while DOC concentrations decreased strongly with depth in the mineral soil solution (Tab. 4.1), explaining rising free Al^{3+} concentrations (Fig. 4.2). However, even in the mineral soil solutions the Al^{3+} concentrations were < 1 μM in 92 % of the samples and the remaining 8 % of the samples with

higher Al^{3+} concentrations ($1 - 6\ \mu M$) would only affect sensitive and moderately sensitive plants (Wheeler et al. 1992).

After addition of 2 times 5 kg ha^{-1} yr^{-1} of Ca (in approximately 6-month intervals) to the Ca treatments since January 2008, in the LL, Ca:Al as well as Mg:Al molar ratios became narrower (Tab. 4.4) relative to the untreated controls. This effect is caused by significantly increased total Al concentrations in the LL of the Ca fertilized plots (Tab. 4.4). The fertilized Ca is nearly completely (97 %) retained in the thick organic horizons and is probably taken up by soil organisms and plants (Wullaert et al. 2013). Accordingly, enhanced Al in LL might be caused by cation exchange of Al^{3+} because of the high amendment of Ca^{2+} (Wullaert et al. 2013) as a kind of reversed acidification process, which will increase acid neutralisation capacity of the organic layers. Another possibility is a decreased Al uptake of plants caused by higher Ca availability and ion competition between Al and Ca. The increased Al concentrations in the LL of the fertilized plots have probably no toxic effects because of the high degree of organic complexation. There was no effect of Ca fertilization on Ca:Al or Mg:Al molar ratios in SS15 and SS30 (Tab. 4.4) because of the lack of significant Ca leaching with the LL. However, Cl^- concentrations and ionic strength are strongly enhanced in the Ca-amended plots at all depths. As indicated by close correlations, the accompanying leached cations were mainly K^+ in LL and SS15 and Na^+ in SS30. This result suggests that K^+ was retained in the upper part of the mineral soil because of the higher exchange capacity of K^+ relative to Na^+, some plant uptake, and K^+ fixation in illites which were reported to occur abundantly in the study area (Schrumpf et al. 2001). In SS15, Mg concentrations and in SS30, Al and Mg concentrations increased significantly in the Ca-amended plots. The simultaneous increase in Al and Mg concentrations explains the absence of a change in Mg:Al molar ratios.

In 73 % of the cases the total Ca:Al molar ratios in our study were < 1, which might indicate possible negative effects on plant growth because of disturbed Ca uptake as suggested in the literature based on research in the temperate zone (Cronan and Grigal 1995). Yet, most of the Al is organically complexed, which renders the Ca^{2+}:Al^{3+} molar ratios far wider (Tab. 4.2) and Al-induced Ca deficiency unlikely.

In the recent study by Rehmus et al. (2014b) on Al toxicity to native tree species from the south Ecuadorian montane forest, impaired Mg uptake at increased Al concentrations was found. Magnesium deficiency and consequently reduced photosynthesis was identified as a possible reason for reduced shoot biomass. Magnesium acts similar to Ca as part of the Ca/Mg-Al antagonism but Mg deficiency might be a more serious problem because of lower Mg concentrations in soil solutions. In the experiment by Rehmus et al. (2014b), a significant decrease in Mg concentrations in plant tissue was recorded at Al concentrations of 600 μM while 300 μM showed no effect. Thus, in the experiment Mg:Al molar ratios of 0.33 showed no effect, while ratios of 0.17 did. Transferring these findings from hydroponic experiments to soil solution would indicate a possible problem of Al-induced Mg deficiency at least in the mineral soil (Tab. 4.1 and 4.2). However, antagonistic interactions are complex and Mg^{2+}:Al^{3+} molar ratios are again far wider.

4.6 Conclusions

i) Modeled and measured concentrations of free Al species in soil solutions showed a good agreement, demonstrating the suitability of modeling approaches to assess Al speciation in DOM-rich soil solutions.

ii) The total and free Al concentrations are in a range in which Al toxicity is unlikely, except for very sensitive plants.

iii) Total dissolved Al concentrations as well as Al^{3+} concentrations are lowest in the organic layer where most plant roots are located. Total Al concentrations increased in the mineral soil solutions, while Al^{3+} concentrations steadily increased from LL to SS30. The changes in Al speciation with depth are mainly attributable to decreasing DOC concentrations.

iv) Calcium amendment of the forest caused the opposite effect as expected. The Ca:Al molar ratios in LL decreased because of increasing Al concentrations while Ca concentrations did not change significantly. The enhanced Al concentrations in LL might be caused by decreased Al uptake of plants be-

cause of higher Ca availability or exchange of Al^{3+} by Ca^{2+} at soil exchange sites and root surfaces.

4.7 Acknowledgments

We thank Jose Luis Peña Caivinagua and Orly Mendoza Aguirre for support in field sampling and maintaining the experiment, Nature and Culture International in Loja, EC, for providing the study area and the research station, Ecuadorian authorities for the research permit, and the German Research Foundation (DFG) for funding (FOR 816, Wi 1601/8-2).

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5. Aluminum cycling in a tropical montane forest ecosystem in southern Ecuador

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5.1 Abstract

Growth limitation induced by aluminum toxicity is believed to commonly occur in tropical forest ecosystems, although a direct proof is lacking. We assessed Al fluxes, drivers of Al concentrations in ecosystem solutions, and indicators of Al toxicity in a tropical montane rainforest in Ecuador in order to test for indications of Al toxicity. We determined Al and Ca concentrations in the leaves of 17 tree species and the base and Al saturation of 23 soil A horizons. High Al fluxes in litterfall (8.77±1.3 – 14.2±1.9 kg ha^{-1} yr^{-1}, mean±SE) indicate a high Al circulation through the ecosystem. The Al concentrations in the organic layer leachate were driven by the acidification of the ecosystem and increased significantly between 1999 and 2008. However, the Ca:Al molar ratios in organic layer leachate and all aboveground ecosystem solutions were above 1, the threshold for Al toxicity. Except for two Al accumulating and one non-accumulating tree species, the Ca:Al molar ratios in tree leaves were above the Al toxicity threshold of 12.5. The fraction of exchangeable and potentially plant available Al in mineral soils was high, being a likely reason for a low root length density in the mineral soil. Our results demonstrate that a wide distribution of Al accumulating tree species and high Al fluxes in the ecosystem do not necessarily imply a general Al phytotoxicity.

5.2 Introduction

Many plant species are sensitive to plant-available Al concentrations in soil solutions, which can occur at pH values < 5.5 and are phytotoxic as Al^{3+}, $AlOH^{2+}$, or $AlOH_2^+$ and in various inorganic but not organic complexes (Alleoni et al. 2010; Delhaize and Ryan 1995; Kabata-Pendias and Pendias 2001; Macdonald and Martin 1988). Aluminum phytotoxicity may contribute to forest decline in temperate forests (Cronan 1989; Godbold et al. 1988; Farr et al. 2009) albeit at soil pH values lower than in the tropics, where pH usually ranges between 4 and 5 controlled by the Al oxide buffer. In tropical montane forests, Al toxicity was suggested to contribute to low biomass production and slow nutrient-cycling rates (Bruijnzeel 2001; Bruijnzeel and Veneklaas 1998; Hafkenscheid 2000). However,

the hypothesis relating the stunted growth of tropical montane forests with Al toxicity was to our knowledge never directly tested by dose-effect experiments with tropical forest trees except for our previous work (Rehmus et al. 2014a,b). Our hydroponic experiment with seedlings of three different tree species typical for the south Ecuadorian montane forests has shown that Al toxicity thresholds for native plant species are higher than reported Al concentrations in soil solutions (10 – 40 μM Al, range of EC10 values for shoot biomass production: 126 to 376 μM Al, Rehmus et al. 2014a).

Seasonal acid deposition originating from Amazonian forest fires (Boy and Wilcke 2008) and the increasing NH_4^+ deposition with subsequent nitrification already in the forest canopy and nitrate leaching through the ecosystem result in acidification of the organic layer leachate of the east Andean tropical montane forests on the rim of the Amazon basin (Wilcke et al. 2013a,b). A continuous and pronounced acidification period of 10 years was observed between two strong La Niña events in 1999/2000 and 2009/2010. During La Niña events, base metal deposition and the pH of the precipitation increase strongly and partly reverse the acidification trend (Boy and Wilcke 2008; Wilcke et al. 2013a). An overal acidification of the ecosystem might lead to increased Al mobilization and an increased threat of Al phytotoxicity.

Knowledge of pH values and Al concentrations in soil solutions alone are not sufficient to judge the threat of Al toxicity, because Al speciation is crucial for toxic effects (Alleoni et al. 2010). In the tropical montane forests on the east-exposed Andean slope of the eastern cordillera, most nutrients are stored in the thick organic layers (Wilcke et al. 2002) where also 51 to 76 % of the fine root length is located (Soethe et al. 2006). Previous studies showed that depending on dissolved organic matter concentrations up to 97 % of the Al in organic layer solution are organically bound in complexes and are nontoxic (Wullaert et al. 2013). Another commonly used approach to estimate the threat of Al-stress to plants is the Ca:Al molar ratio in plant tissue and soil solution and the base saturation of the soil (Cronan and Grigal 1995), because the Ca-Al antagonism may disturb the Ca nutrition at high Al concentrations (Rengel 1992). According to Cronan and Grigal (1995), indices for a 50 % risk of adverse impacts on tree growth induced by Al stress are a Ca:Al molar ratio ≤ 1.0, ≤ 0.2, and ≤ 12.5 in soil

solution, fine root tissue, and foliar tissue, respectively, and a soil base saturation $\leq$ 15 % of the effective cation exchange capacity (ECEC). Aluminum can also affect the Mg nutrition of plants (Kidd and Proctor 2000; Kinraide 2003). De Wit et al. (2010) found reduced Mg concentrations in needles of *Picea abies* in an in-situ experiment with elevated Al concentrations (up to 500 μM) in soil solution. In our previous hydroponic experiment we revealed impaired Mg translocation to the leaves and possibly reduced photosynthesis as a reason for reduced shoot biomass production under Al stress (Rehmus et al. 2014b).

The overall aim of this study was to assess the general assumption of Al phytotoxicity in tropical montane forests in southern Ecuador.

Our objectives were to

i) quantify all major Al fluxes and to calculate the Al budget of a 9-ha large catchment

ii) identify the drivers of Al concentrations in ecosystem solutions, i.e. the influence of acidification and presence of (organic) ligands, and

iii) assess Al toxicity to plants by the combined use of different indicators including Ca:Al molar ratios in leaves and soil solution and base saturation of the cation exchange sites in mineral soil.

We used existing data from a long-term ecosystem study which were complemented with additional measurements.

5.3 Materials & Methods

5.3.1 Study site and sampling procedures

The study site is an approx. 9 ha-large microcatchment (MC 2) between 1900 and 2010 m a.s.l. on 30 – 50° steep slopes on the north-facing part of the Rio San Francisco valley (see Boy and Wilcke (2008) for a map of the study site). The ecosystem flux measurements in the forest are concentrated along three ca. 20 m-long transects covering about 10 m in elevational difference at 1900 – 1910, 1950 – 1960 and 2000 – 2010 m a.s.l. (MC 2.1, MC 2.2, and MC 2.3, Fig. 5.1).

The underlying bedrock is interbedding of palaeozoic phyllites, quartzites, and metasandstones, from which mainly Dystric Cambisols developed; at higher positions Folic Histosols can be found (IUSS Working Group WRB 2007). However, the Histosols did not originate from high groundwater levels but were formed by thick and largely well aerated organic layers because of high litterfall rates (ca 10 kg ha^{-1} yr^{-1}) and slow organic matter turnover (Wilcke et al. 2002).

In the period January 1998 to December 2010, mean annual precipitation was 1950 mm. June tended to be the wettest month, with 240 mm of precipitation on average, in contrast to 116 mm in November, the driest month. The mean temperature at 1950 m above sea level was 16.0°C. The coldest month was July, with a mean temperature of 14.5°C; the warmest was November, with a mean temperature of 16.8°C (personal communication Thorsten Peters, Paul Emck, Rütger Rollenbeck, Jörg Bendix, and Michael Richter).

Incident precipitation was collected with Hellmann-type collectors, first at one clearing in the study area, in May 2000 extended to 3 measurement stations, each equipped with five samplers. Throughfall precipitation was first collected with five, since Mai 2000 with eight, and since Mai 2003 with twenty Hellmann-type collectors at each of the three measurement transects (MC 2.1 – 2.3). Stemflow was collected with a polyurethane foam collar on five representative large trees in MC 2.1 and surface flow was measured with a V-shaped weir at the outlet of the stream drainage MC 2. The weir collected all water drained from the whole catchment (Fleischbein et al. 2006). There were no indications that the catchment was not dense or received additional water from outside (Goller et al. 2005; Fleischbein et al. 2006). Furthermore, surface flow outside the stream never occurred (own observation). Water levels at the weir were recorded hourly with a pressure gauge and additionally measured manually after the stream water samples were collected. Suspended matter in river export was volume-sampled twice weekly between 2001 and 2003 at the V-notch weir (250 mL runoff water per sampling). Suspended matter was concentrated by accumulative filtering of the stored solutions at 0.45 μm pore size (3 months runoff per single filter).

Further instrumentation (to collect litter leachate, soil solutions, and litterfall) was placed along three measurement transects (MC 2.1 – 2.3). The transects were considered representative for the whole catchment. Because the whole catchment

was below the condensation level at 2200 m a.s.l. (Bendix et al. 2006), there was no significant difference in throughfall and litterfall among the three transects. Soil solution was collected with three zero-tension lysimeters below the organic layer (organic layer leachate (LL) below Oi, Oe, and Oa horizons) and with three suction cups at 0.15 m and 0.30 m depths (SS15 and SS30, respectively) in the mineral soil of each of the three transects. The lysimeters were introduced from the wall of soil pit to minimize disturbance of the organic layer. The lysimeters were pushed ca. 0.50 m into the soil so that they were entirely covered by the organic layer. To avoid adsorption/desorption effects in soil solution, a vacuum was applied on the suction cups after installation allowing for equilibration of the cups and the soil solution of the first month was discarded. According to Guggenberger and Zech (1992), equilibrated ceramic suction cups do not show significant interactions with the soil solution.

Weekly sample replicates were bulked to a composite sample per measurement site prior to chemical characterization. Soil water content measurements with FDR (frequency domain reflectometry) probes at transect MC 2.1 at the 0.1, 0.2, 0.3, and 0.4 m depths were used for calculation of soil water fluxes of the three sampling sites (MC 2.1 – MC 2.3, Fleischbein et al. 2006).

Each of the three measurement transects was equipped with three litter collectors with the dimension 0.3 m x 0.3 m and 0.5 mm mesh size. Samples were collected weekly and dried to constant mass at 40°C in an oven. The sample mass was recorded for each collector separately; the samples were then bulked to a monthly sample per microcatchment which was chemically characterized.

Subject of the present investigation were in general the monthly means from April 1998 to March 2003. In the case of soil solutions, the investigated time interval was May 2000, when soil solutions were sampled for the first time, to April 2003. The organic layer leachate was analyzed from April 1998 to December 2007 and September 2009 to April 2010, with smaller gaps (6 %) in between due to missing samples.

Samples of fresh tree leaves representing the most abundant tree species from the study area were collected in two sampling campaigns between October 2005 and February 2006 and in October 2011 from 21 and 9 individual trees, respectively (17 tree species in total, Tab. 5.1).

The base and Al saturation of the cation exchange capacities of the mineral soils was determined in the A horizons of 23 soils. Three of the soil samples (collected at our measurement transects) were composited from three separate samplings with soil pits at the lower and upper ends and in the middle of the transects MC 2.1 to MC 2.3, 10 soils were located near the stream draining the watershed between 1880 and 2100 m a.s.l. (valley bottom) and further 10 soils were located near the ridge between 1890 and 2110 m a.s.l. We considered the selected data set of soils as representative for the whole catchment.

5.3.2 Chemical analyses

Concentrations of Cl^- in ecosystem solutions were determined with a Cl^--specific ion electrode (Orion 9617 BN, Thermo Fisher Scientific, Waltham, USA) immediately after collection in Ecuador during the first 3 years. In the fourth and fifth year, Cl^- was analyzed with a segmented Continuous Flow Analyzer (CFA, San plus, Skalar, Breda, Netherlands). Total organic carbon (TOC) concentrations were analyzed with a TOC-5050 (Shimadzu, Düsseldorf, Germany). The Ca, Mg, K, and Na concentrations were determined with atomic absorption spectroscopy (AAS, SpectraAA400, Varian, Darmstadt, Germany and Zeenit700P, Analytik Jena, Jena, Germany), the Al concentrations with inductively coupled plasma mass spectrometry (ICP-MS, VG PlasmaQuad PG2 Turbo Plus, Thermo Fisher Scientific, Waltham, USA and 7700x Agilent Technologies, Santa Clara, CA).

Litter- and leaf samples were digested with 65 % HNO_3 (leaf samples of 2005/2006) or 69 % HNO_3/ 30 % H_2O_2/ 48 % HF/ 5 % H_3BO_3 (leaf samples of 2011) in a closed vessel microwave system (MARS Xpress, Kamp-Lintfort, Germany and MLS Ethos, Leutkirch, Germany, respectively) after drying and homogenization with a ball mill. Calcium, Mg and Al in leaf digests from the first sampling were analyzed with AAS (AA240FS, Varian, Darmstadt, Germany). In leaf digests from the second sampling Ca and Mg were analyzed with AAS (Zeenit 700P, Analytik Jena, Jena, Germany) and Al with ICP-MS 7700X (Agilent Technologies, Santa Clara, CA). Concentrates of particulate matter from the stream water were digested with 65 % HNO_3/ 48 % HF and measured by AAS for metal content.

The base saturation in A horizons of the soils was determined by extraction with 1 M NH_4NO_3 (soil:solution ratio 1:25). Calcium, K, Mg, Na, Al, Fe, and Mn were determined with AAS (SpectrAA400, Varian, Darmstadt, Germany). The pH values were determined in 0.01 M $CaCl_2$ (soil:solution ratio 1:2.5) with a standard pH electrode (Orion U402-S7, Thermo Fisher Scientific, Waltham, USA).

5.3.3 Calculations and statistical evaluation

The base saturation and saturation of exchangeable Al was calculated as the proportion of charge equivalent of extractable Ca + K + Mg + Na and Al of the ECEC. To test if Al concentrations in organic layer leachate followed a temporal trend from 1998 to 2010, a Seasonal Mann-Kendall test which accounts for seasonal autocorrelation of the data was conducted. Trends in monthly Al concentrations were tested for the whole investigation period and additionally starting from the La Niña event in 1999 until the end of the continuous acidification period in December 2007.

The Al fluxes were calculated for 5 consecutive hydrological years from April 1998 to March 2003 (in the case of soil solutions 3 years from May 2000 to April 2003). Data gaps in surface flow caused by logger breakdowns were replaced by modeled values obtained with the hydrological modelling program TOPMODEL (Beven et al. 1995) as described in Fleischbein et al. (2006). Data gaps of soil water fluxes (because of lacking soil water contents) were substituted with the help of a regression model of weekly soil water fluxes on weekly throughfall volumes (R^2 = 0.85).

The Al canopy budget and total Al deposition was estimated with the canopy budget model of Ulrich (1983) using Cl^- as inert tracer (see Boy and Wilcke (2008) for a detailed description). Annual Al and Cl fluxes were calculated based on monthly mean concentrations averaged from usually 4 – 5 weekly measurements. If one monthly concentration per year was missing, which was the case for Al in litterfall at all three measurement transects in November 1999, in August 2002 at MC 2.3 in SS15 and SS30, and in February 2003 at MC 2.2 in SS15 and SS30, the value was substituted by the mean of the previous and the consec-

utive monthly concentration. If more than one monthly concentration per year was missing, which was the case for Cl in rainfall, throughfall at all three measurement transects, and stemflow in the years 2000 and 2001, the annual fluxes were calculated with volume-weighted means. The dissolved Al catchment budget was calculated by subtracting the annual total deposition (i.e. bulk and fine particulate dry deposition) from aluminum fluxes in stream water.

We performed multiple regressions among monthly Al concentrations and Ca:Al molar ratios, respectively, with pH values and TOC concentrations in the soil solutions, and additionally with soil moisture in case of the organic layer leachate. The soil moisture was considered because it influences the Al concentrations via concentration/dilution effects. Regression residuals were tested for normal distribution with the Shapiro-Wilk test and for homogeneity of variances with the Breusch-Pagan test. Only variables with a significant influence on Al concentrations were used in the resulting linear model. We square-root transformed Al concentrations in organic layer leachate and the soil solution at 0.30 m soil depth and Ca:Al molar ratios in soil solution at the 0.15 m and 0.30 m depth, because of a data skewness > 0.5 (Webster 2001). The Ca:Al molar ratios in organic layer leachate and TOC concentrations in the soil solution at 0.30 m depth were log-transformed because of a data skewness > 1. Furthermore, we eliminated outliers (values greater than 1.5 times the interquartile range) in Ca:Al molar ratios of organic layer leachate ($n = 4$) and Al concentrations ($n = 1$) and Ca:Al molar ratios ($n = 1$) in the soil solution at 0.30 m depth. Statistical analyses were carried out with R 3.0.2 (R Core Team 2013).

5.4 Results

5.4.1 Al concentrations and fluxes

The highest Al concentrations occurred in SS15, followed by SS30 and organic layer leachate and decreased in each of the solutions from MC 2.1 to MC 2.3 (Tab. 5.2). Aluminum concentrations in precipitation, stemflow, and stream water were below 1 μM.

Table 5.1: Species and family name, number of individual replicates (n), Ca, Mg, and Al concentrations (mg g^{-1}) and Ca:Al molar ratios in leaves sampled from trees in the study area in south Ecuador between October 2005 and February 2006 (García Ochoa 2007) and in October 2011 and in litterfall from April 1998 to March 2003

species	family	n	Ca	Mg	Al	Ca:Al
				mg g^{-1}		molar ratio
sampling October 2005 to February 2006						
Purdiaea nutans Planch.	Cyrilliaceae	3	9.8 ± 0.8	2.6 ± 0.3	0.1 ± 0.0	53.7 ± 7.2
Alchornea pearcei Britton.	Euphorbiaceae	3	6.6 ± 1.9	1.9 ± 0.3	0.1 ± 0.0	64.0 ± 16.5
Graffenrieda emarginata (Ruiz & Pav.)	Melastomataceae	3	2.4 ± 0.2	1.6 ± 0.2	3.4 ± 0.4	0.5 ± 0.1
Podocarpus oleifolius (Donex Lamb.)	Podocarpaceae	3	4.3 ± 0.7	1.4 ± 0.2	0.1 ± 0.0	37.1 ± 1.3
Alazatea verticilata (Ruiz & Pav.)	Lythraceae	1	4.7	1.8	0.07	47.9
Clusia ducoides (Engl.)	Clusiaceae	1	10.9	2.0	0.05	144
Hyeronima moritziana (Mull. Arg.)	Euphorbiaceae	1	3.9	1.5	0.09	30.2
Ocotea aciphylla (Nees) Mez.	Lauraceae	1	2.5	1.2	0.1	19.8
Ocotea bentamiana Mez.	Lauraceae	1	1.7	1.2	0.12	9.2
Miconia sp	Melastomataceae	1	7.7	3.3	1.64	3.2
Elaeagia sp	Rubiaceae	1	3.7	1.6	0.10	25.4
Matayba inelegans Spruce ex Radlk.	Sapindaceae	1	3.2	2.7	0.06	34.8
Prunas opaca (Benth.) Walp.	Rosaceae	1	12.5	2.6	0.09	99.0
sampling October 2011						
Cedrela odorata L.	Meliaceae	1	6.5	2.3	0.10	42.9
Cedrela sp	Meliaceae	2	9.1 ± 2.8	3.3 ± 0.3	0.12 ± 0.02	47.6 ± 9.5
Heliocarpus americanus L.	Tiliaceae	3	7.6 ± 1.4	5.3 ± 0.7	0.14 ± 0.04	42.9 ± 13.3
Tabebuia chrysantha (Jacq.) G. Nicholson	Bignoniaceae	3	5.8 ± 1.3	3.2 ± 0.4	0.07 ± 0.01	53.4 ± 11.5
litterfall			12.1 ± 0.2	4.00 ± 0.1	1.15 ± 0.06	8.5 ± 0.6

Values are means ± SE of individual replicates

Table 5.2: Range and mean of pH, mean Ca (range published in Wilcke et al. 2008), Mg, and Al concentrations (μM), Ca:Al and Mg:Al molar ratios, TOC concentrations (mg L^{-1}), and soil moisture (vol. %) in bulk precipitation, throughfall, stemflow, organic layer leachate (LL), soil solutions in 0.15 m and 0.30 m depth (SS15 and SS30, respectively), and stream water in MC 2.1, MC 2.2, and MC 2.3 from April 1998 to March 2003 (LL April 1998 to April 2010) and in soil solutions from May 2000 to April 2003

solution	pH		Ca	Mg	Al	Ca:Al	Mg:Al	TOC	soil moisture
	range	mean		μM		molar ratio		mg L^{-1}	vol. %
precipitation MC 2	3.9 – 7.8	5.1	6.44 ± 0.62	4.44 ± 0.47	0.33 ± 0.04	21.3 ± 2.9	15.8 ± 2.9	5.28 ± 0.18	
throughfall MC 2.1	4.5 – 8.2	6.2	25.1 ± 1.7	20.7 ± 1.3	1.39 ± 0.08	23.6 ± 2.0	18.4 ± 1.5	1.12 ± 0.03	
throughfall MC 2.2	4.9 – 7.5	6.2	22.9 ± 1.2	20.4 ± 1.1	1.30 ± 0.08	24.1 ± 1.8	21.4 ± 1.6	1.19 ± 0.04	
throughfall MC 2.3	4.2 – 7.8	6.0	41.9 ± 1.7	43.1 ± 2.0	1.47 ± 0.09	35.8 ± 2.1	36.5 ± 2.4	1.44 ± 0.06	
stemflow MC 2.1	4.2 – 7.7	6.1	26.3 ± 1.4	22.0 ± 1.2	0.99 ± 0.07	46.7 ± 4.7	37.3 ± 4.0	18.3 ± 0.5	
stream water MC 2	5.5 – 9.1	6.7	16.6 ± 0.7	18.2 ± 0.6	0.47 ± 0.07	48.6 ± 3.5	59.7 ± 4.7	4.48 ± 0.28	
LL MC 2.1	3.8 – 6.6	4.4	31.9 ± 1.6	45.5 ± 1.9	27.18 ± 0.8	4.74 ± 1.4	4.21 ± 1.13	3.37 ± 0.08	17.8 ± 0.6
LL MC 2.2	3.6 – 7.5	4.6	48.8 ± 2.5	58.2 ± 3.0	14.3 ± 0.9	10.4 ± 3.60	9.24 ± 2.42	2.81 ± 0.10	n.d.
LL MC 2.3	3.3 – 6.9	5.0	111 ± 5.2	89.7 ± 4.8	4.42 ± 0.36	42.1 ± 3.7	34.2 ± 3.2	3.84 ± 0.19	n.d.
SS15 MC 2.1	3.8 – 4.6	4.2	7.99 ± 1.20	7.14 ± 0.92	73.0 ± 3.02	0.12 ± 0.02	0.10 ± 0.01	2.25 ± 0.05	n.d.
SS15 MC 2.2	4.0 – 5.6	4.4	6.19 ± 0.77	24.3 ± 1.9	12.4 ± 0.94	0.60 ± 0.08	2.15 ± 0.13	0.64 ± 0.02	n.d.
SS15 MC 2.3	4.4 – 5.2	4.9	31.6 ± 1.9	16.6 ± 1.4	9.35 ± 0.32	3.60 ± 0.27	1.86 ± 0.16	0.89 ± 0.04	n.d.
SS30 MC 2.1	4.1 – 4.9	4.4	3.53 ± 0.50	3.70 ± 0.55	36.8 ± 1.9	0.10 ± 0.01	0.10 ± 0.01	1.29 ± 0.04	n.d.
SS30 MC 2.2	4.2 – 5.6	4.5	8.71 ± 1.08	24.7 ± 3.5	13.3 ± 1.4	0.80 ± 0.10	2.07 ± 0.20	0.64 ± 0.03	n.d.
SS30 MC 2.3	4.4 – 5.6	5.2	40.6 ± 11.4	21.2 ± 5.0	4.86 ± 0.28	6.64 ± 0.58	3.58 ± 0.42	0.68 ± 0.07	n.d.

± SE
n.d. is not determined

Aluminum concentrations in leaves of the tree species from the study area were generally in the range of 0.05 – 0.14 (mg g^{-1}) (Tab. 5.1). Two tree species, *Graffenrieda emarginata* (Ruiz & Pav.) and *Miconia sp*, which belong to the family of Melastomataceae and are Al accumulators (Jansen et al. 2002), had distinctly higher Al concentrations than the Al non-accumulating tree species.

Mean annual Al fluxes at each of the three measurement transects were lowest in bulk, dry, and throughfall deposition (< 0.6 kg ha^{-1} yr^{-1}, Fig. 5.1). Stemflow contributed negligibly to the Al fluxes reaching the soil (< 0.05 – 5.4 %). Deposition by litterfall was 9 to 18 times higher than the sum of all dissolved aboveground Al fluxes. The mean annual litter input at MC 2 from April 1998 to March 2003 was 9.99±0.27 t ha^{-1} yr^{-1}. The mean annual canopy budget was positive at each of the three measurement transects. Belowground, Al fluxes decreased from transect MC 2.1 to MC 2.3, i.e. with increasing elevation. At transects MC 2.1 and MC 2.2, the Al fluxes in organic layer leachate were lower than in mineral soil solutions. At transect MC 2.3, lowest Al fluxes occurred in SS30. The catchment budget of dissolved Al was negative, yet the loss of Al as particulate matter averaged 28.8 kg ha^{-1} yr^{-1} (August 2000 – January 2003).

The study by Wullaert et al. (2013) had shown that only TOC occurred in relevant concentrations for Al complexation. Complexes of other ligands (Cl^-, F^-, NO_3^-, PO_4^{3-}, SO_4^{2-}) hardly contributed to total Al concentrations. These previous findings were confirmed by the results presented in Chapter 5.

5.4.2 Drivers of Al concentrations

Potential drivers of Al concentrations are soil acidity and concentrations of complexing agents including TOC, F^-, and SO_4^{-2} (Tab. 5.2). Furthermore, total Al concentrations are influenced by dilution/concentration effects which we addressed by including soil moisture in our analysis.

The multiple regression among Al concentrations (C_{Al}) and pH values, soil moisture, and TOC concentrations (C_{TOC}) in organic layer leachate ($R^2 = 0.31$, $p < 0.001$, $n = 75$) is shown in Equation 5.1.

$$C_{Al} = 7.88 - 0.88 \cdot pH - 0.03 \cdot soilmoisture(vol.\%) + 0.02 \cdot C_{TOC}(mgL^{-1}) \quad (5.1)$$

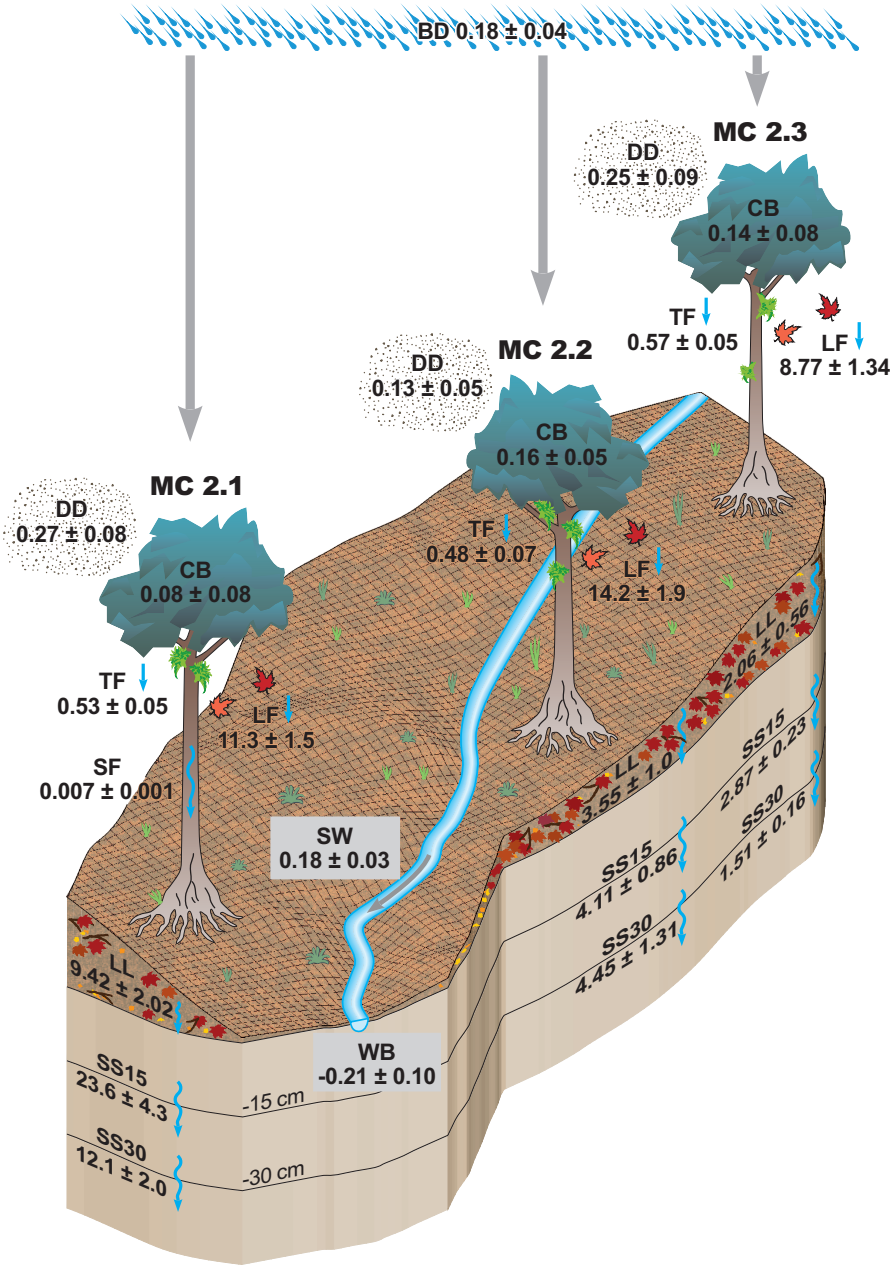

Figure 5.1: Schematic illustration of Al fluxes (kg ha^{-1} yr^{-1}) in bulk (BD) and dry deposition (DD), throughfall (TF), litterfall (LF), stemflow (SF), organic layer leachate (LL), soil solution in 0.15 m (SS15) and 0.30 m (SS30) soil depth, and stream water (SW), and the Al canopy (CB) and dissolved Al catchment budgets (WB). Values are arithmetic means of annual values (± SE) from 1998 to 2003 (n = 5), in case of soil solutions from 2000 to 2003 (n = 3)

The highest regression coefficient was achieved for the pH value which shows that the pH has the greatest influence on Al concentrations of the tested variables (Fig. 5.2). Only the pH values had a significant influence on Ca:Al molar ratios in LL (Ca:Al molar ratio = -0.75 + 0.27 · pH, $R^2 = 0.12$, $p < 0.001$, n = 111). There was no significant relationship among Al concentrations or Ca:Al molar ratios and pH values and TOC concentrations in the soil solutions in the 0.15 m and 0.30 m soil depth.

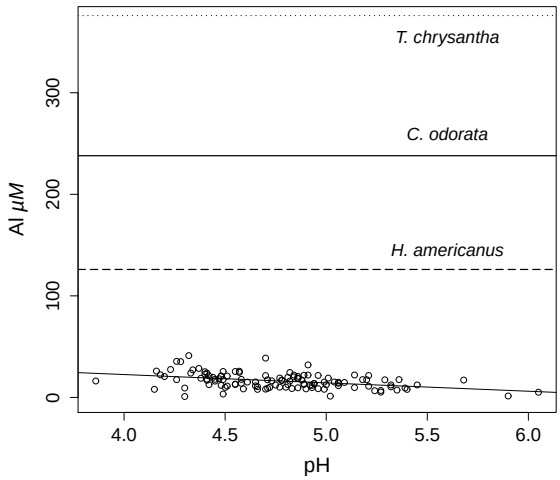

Figure 5.2: Relationship between pH values and Al concentrations (monthly means) in organic layer leachate from April 1998 to April 2010. The trendline is shown for illustration purposes. The dotted, continuous, and dashed line illustrates the EC10 values on shoot biomass production, i.e. the Al concentration at which 10 % reduction occured, for seedlings of *T. chrysantha*, *C. odorata*, and *H. americanus*, respectively (Rehmus et al. 2014a)

A seasonal Mann-Kendall test confirmed significantly increasing Al concentrations in organic layer leachate from April 1998 untill April 2010 (Fig. 5.3). When tested for the period of acidification of the organic layer leachate from July 1999 to December 2007, starting from the time of base metal input and high pH values during La Niña, the trend was even closer ($\tau = 0.38$, $p < 0.001$).

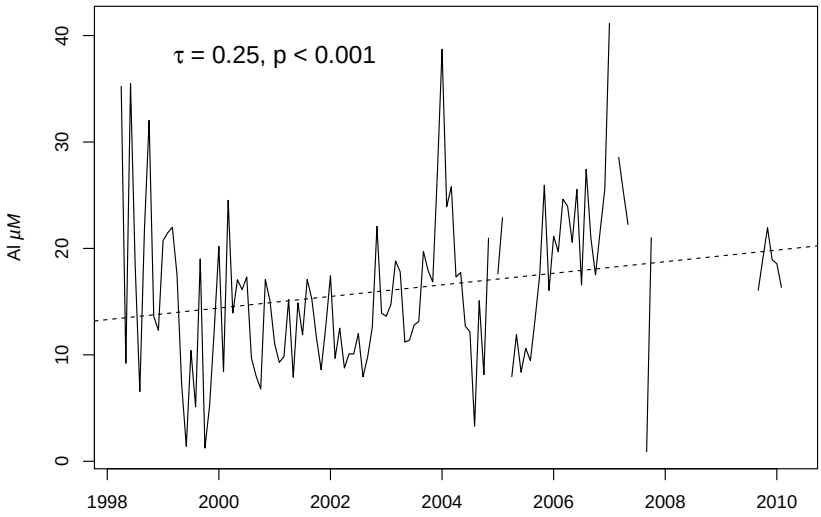

Figure 5.3: Monthly means of Al concentrations (μM) in organic layer leachate from April 1998 to April 2010. The trendline is shown for illustration purposes. The trend is significant according to the seasonal Mann-Kendall test

5.4.3 Toxicity indicators

The Ca:Al molar ratios of ecosystem solutions were lowest in SS15, followed by SS30 and increased from MC 2.1 to MC 2.3. The Ca:Al molar ratios in the other ecosystem solutions were distinctly higher (Tab. 5.2).

The Ca and Mg concentrations in the leaves of the investigated tree species from the study area ranged 1.7 – 12.5 and 1.2 – 5.3 (mg g^{-1}), respectively (Tab. 5.1). The Ca:Al molar ratios were consistently higher than 12.5 (indicating potential toxicity) and ranged up to 144. Only the two already mentioned Melastomataceae species and *Ocotea bentamiana* Mez. (Lauraceae) had Ca:Al molar ratios ranging 0.5 – 9.2.

The mean pH ($CaCl_2$) determined from H^+ activities and back transformed to pH in soil A horizons was acid (Tab. 5.3). The base saturation and correspondingly the Al saturation had a wide range. Seven of the 23 soil A horizons had a base saturation of ECEC below 15 % and two soil A horizons had a base saturation between 15 and 16 % (Fig. 5.4). Soils with low base saturation mainly occurred on the ridges (mean±SE base saturation on the ridges: 22.2±5.2 % and in the valley:

65.2±9.1 %) while there was no elevational effect on base saturation (Wilcke et al. 2010). The base saturation increased and Al saturation decreased from MC 2.1 to MC 2.3 (Wilcke et al. 2001).

Table 5.3: Descriptive statistics of pH ($CaCl_2$), base saturation (BS), and Al saturation (AlS) in A horizons of 23 soil profiles from MC 2

Property	Mean	Standard Error	Median	Min	Max
pH ($CaCl_2$)	3.74	-	4.01	3.19	5.17
BS*	41.38	6.74	31.71	4.77	97.08
AlS*	57.73	6.71	68.29	2.65	95.21

* in % of ECEC

5.5 Discussion

5.5.1 Al fluxes

The Al fluxes with bulk and dry deposition were low compared to other tropical rain forests in Brazil (Mayer et al. 2000; Cornu et al. 1998) and a temperate forest in Germany, which was affected by acid deposition (Matzner 1989, Fig. 5.1 and Tab. 5.4). The Al bulk deposition resembled a temperate forest in the USA (Rustad and Cronan 1995) and was higher than the Al bulk deposition in the Hubbard Brook experimental forest (Likens 2013) and a humid tropical ecosystem in Fiji during wet and dry season excluding cyclone events (Waterloo et al. 1997), during which Al concentrations were below the detection limit because of strong dilution. Aluminum deposition from the atmosphere mostly derives from mineral dust, because Al is an ubiquitous element in soils, occurring mostly as alumosilicates (Macdonald and Martin 1988). The Al deposition with throughfall resembled that of a tropical lowland forest in Brazil (Cornu et al. 1998) and was higher than in a temperate forest in the USA (Rustad and Cronan 1995) but three to six times lower than in a temperate forest in Germany (Matzner 1989) and a costal rain forest in Brazil (Mayer et al. 2000). The positive canopy budget indi-

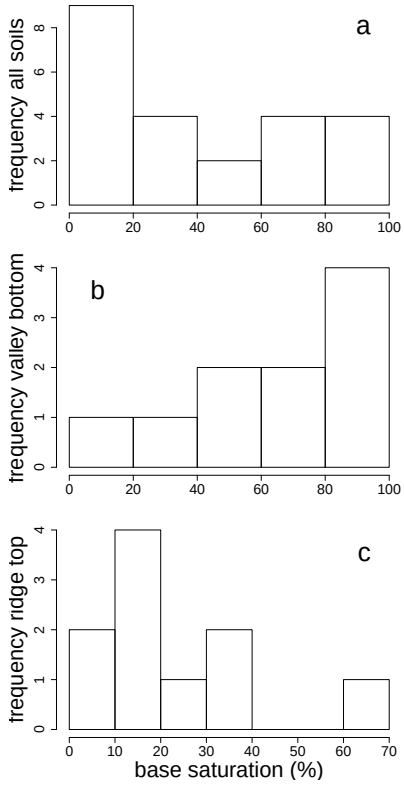

Figure 5.4: Frequency histogram of base saturation (% of ECEC) of 23 A horizons combined (a), of the 10 A horizons from the valley bottom (b), and of 10 A horizons from the ridge top (c) from a 9-ha large catchment (MC 2)

cates leaching of Al from the canopy. The leaching of cations is a result of proton buffering in the canopy (increased pH values of throughfall compared to precipitation, Tab. 5.2) and release of cations to achieve electroneutrality (Matzner 1989). The lowest Al fluxes occurred in stemflow and were lower than in other studies (Tab. 5.4). The input of H^+ and other ions by the stemflow to the soil is restricted to a small area around the stem basis which might have a considerable impact on the soil chemistry and plant growth (Koch and Matzner 1993; Levia and Frost 2003). In our study, the Al concentrations and fluxes in the stemflow were low and pH values were high compared to other ecosystem compartments (Fig. 5.1), indicating little influence on soil acidification and Al availability to plants.

The Al fluxes with litterfall were high compared to temperate forest ecosystems and most study sites in the tropics (Tab. 5.4) and are attributable to both, the overall high quantity of litterfall and to high Al concentrations in litterfall (Tab. 5.1 and Fig. 5.1). The mean annual litterfall input on the present study sites was two and approximately five times that of a deciduous and a coniferous forest in Bavaria, respectively (Berg and Gerstberger 2004). The mean Al concentrations in litterfall were approximately 10 times that of the Bavarian forests. The total litter input and Al fluxes in litter of a lower montane forest in Bolivia at a similar altitude were approximately comparable to that of our study (Tab. 5.4, Gerold 2008). In a tropical montane forest in Jamaica at a slightly lower altitude, the total litter input as well as the Al fluxes in litterfall were lower compared to our study (Tab. 5.4, Hafkenscheid 2000). The Al concentrations in litterfall in the present study were three times higher than the mean Al concentrations in fresh foliage (Tab. 5.1). Hafkenscheid (2000) detected enriched Al concentrations in litterfall compared to fresh leaves of the same species in a tropical montane forest in Jamaica and explained the Al enrichment as an attempt of the trees to dispose excess Al via litterfall. Another reason for Al accumulation in litterfall relative to fresh leaves is the withdrawal of mobile essential nutrients prior to leaf fall.

The Al fluxes with organic layer leachate and soil solutions at our measurement transects had a wide range, covering most of the range reported by other studies from similar soil depths (Fig. 5.1 and Tab. 5.4). The decreasing Al concentrations and fluxes in LL, SS15, and SS30 with elevation are consistent with increasing

pH values of the respective solutions and might be attributable to increasing waterlogging with increasing elevation potentially producing increasing alkalinity with increasing elevation.

In stream water, the dissolved Al fluxes were low compared to two temperate forest ecosystems in the USA (Likens 2013; Rustad and Cronan 1995). The low dissolved Al concentrations are attributable to the high pH of the stream water (Tab. 5.2), causing precipitation of Al e.g., as Al hydroxide. Accordingly, particulate Al was high and the Al loss as particulate matter was 20 times higher than e.g., that of the Hubbard Brook experimental forest (Likens 2013). However, particulate matter mainly originated from soil erosion and not from precipitation of Al oxides in the stream water because dissolved Al concentrations were too low to explain particulate Al concentrations.

Table 5.4: Aluminum fluxes (kg ha^{-1} yr^{-1}) of bulk (BD) and dry deposition (DD), throughfall (TF), stemflow (SF), litterfall (LF*), organic layer leachate (LL), mineral soil solution (SS**), stream water (SW), and particulate loss (PL) from the literature

		Al fluxes (kg ha^{-1} yr^{-1})								
reference	ecosystem	BD	DD	TF	SF	LF*	LL	SS**	SW	PL
Likens (2013)	northern hardwood forest, USA	< 0.01							2.79	1.38
Rustad and Cronan (1995)	northern red spruce forest, USA	0.2		0.06	0.03	0.65	2.1		2.6	
Berg and Gerstberger (2004)	deciduous forest, Bavaria, Germany					0.98 (5.45)				
Matzner et al. (2004)	deciduous forest, Bavaria, Germany							2.4 (0.6)		
Berg and Gerstberger (2004)	coniferous forest, Bavaria, Germany					1.2 (2.14)				
Matzner et al. (2004)	coniferous forest, Bavaria, Germany							17.2 – 26.9 (0.2 – 0.9)		
Matzner (1989)	temperate forest, Solling, Germany	1.2	0.9 – 1.3	1.6 – 2.9				17.6 – 52.7 (0.9)		
Cornu et al. (1998)	tropical lowland rainforest, Brazil	1.4		0.6	0.03	2.62		3.7 (0.4)		
Mayer et al. (2000)	rain forest, Brazil	5.2		3.2				26.5 – 43.5 (0.1 – 1)		
Hafkenscheid (2000)	tropical montane forest, Jamaica					1.50 – 5.22 (6.47 – 6.16)	1.9 – 4.9	16.1 – 35.1 (0.05 – 0.14)		
Gerold (2008)	tropical montane forest, Bolivia					15.5 (12.2)				
Bergamini Scheer et al. (2011)	atlantic rain forest, Brazil					11.2 – 9.69 (6.37 – 3.01)				
Moraes et al. (1999)	atlantic rain forest, Brazil					5.3 (6.31)				

* total litterfall (t ha^{-1} yr^{-1}) in parentheses
** soil depth (m) in parentheses

5.5.2 Drivers of Al concentrations

The mean pH values in organic layer leachate and soil solutions were consistently <5.5 and thus elevated Al concentrations could potentially occur (Tab. 5.2). At our measurement transects, the mineral soil solutions which had the highest Al concentrations play a minor role with respect to Al toxicity, because 51 to 76 % of the fine root length is concentrated in the organic layer (Soethe et al. 2006). On the other hand, this distribution of root length density may be a reaction to the higher Al concentrations in mineral soil solution than in organic layer leachate.

The influence of soil moisture and TOC concentrations on Al concentrations in organic layer leachate was smaller than that of pH values (Equation 5.1). The relationship between pH values and Al concentrations in soil solution of strongly acid soils is exponential at low pH values (Mulder 1988). However, at the pH range of the organic layer leachate in our study the relationship between pH and Al concentration is still in the linear range, leading to moderate changes in Al concentration as response to changes in pH. Furthermore, the solubility of organic matter decreases with decreasing pH and the complexation of Al is reduced, which might shift the solubility equilibrium and decrease total dissolved Al concentrations. However, the Al solubility due to increasing H^+ activity is more pronounced than the latter described effect. In the organic layer leachate, a close negative relationship between pH values and Al concentrations was observed, which confirms the risk of increasing Al mobilization with the acidification of the ecosystem (Fig. 5.2). The trend of increasing Al concentrations during the period 1998 to 2010 was significant despite the deposition of base metals in 1999/2000 and 2009/2010 as a consequence of Sahara dust input (Boy and Wilcke 2008; Wilcke et al. 2013a). The Al concentrations which caused a 10 % reduction in shoot biomass (EC10 values: 126 to 376 μM Al) in the experiment by Rehmus et al. (2014a) were not approached even during strongest acidification of the organic layer leachate (Fig. 5.1 and 5.3). To reach the EC10 values, an approximately 8 to 23-fold increase of the mean Al concentrations in organic layer leachate would be necessary. A review by Schaedle et al. (1989) gives an overview of the response of tree seedlings to Al concentrations in hydroponic and sand/soil culture experiments. The lowest Al concentrations in solution experi-

ments at which a negative response by sensitive species was observed ranged 35 to 170 μM. Compared to the results given by Rehmus et al. (2014a) and Schaedle et al. (1989), the present Al concentrations in the organic layer leachate can be considered as nontoxic.

5.5.3 Toxicity indicators

The speciation of Al should be taken into account when estimating the risk of Al toxicity. Several studies demonstrated an alleviation of Al toxicity by formation of organo-Al complexes with dissolved organic matter in solution (Alleoni et al. 2010; Drabek et al. 2005; Hernandez-Soriano et al. 2013; Vieira et al. 2009). Wullaert et al. (2013) modeled Al speciation at the nutrient manipulation experiment site close to MC2 and found that up to 97 % of the Al in the organic layer solution is organically bound (results confirmed in Chapter 5). The remaining free Al^{3+} concentration in the organic layer leachate of approximately 0.5 μM is unproblematic even for Al-sensitive plant species (Wheeler et al. 1992).

Applying the Ca:Al molar ratio thresholds suggested by Cronan and Grigal (1995), the low Ca:Al molar ratios in SS15 and SS30 from MC 2.1 and MC 2.2 might pose a 50 % and higher risk of Al stress (Tab. 5.2). However, the majority of the tree roots is concentrated in the organic layer which has Ca:Al molar ratios above the toxicity threshold of 1 (Tab. 5.2) indicating no threat of Al toxicity. Furthermore, Al has a higher affinity to dissolved organic matter compared to Ca, causing a free Ca^{2+}:Al^{3+} ion activity ratio of > 60 (Wullaert et al. 2013) which renders an Al effect on plant Ca uptake unlikely. Jorns and Hecht-Buchholz (1985) reported Mg deficiency symptoms in Norway spruce (*Picea abies* (L.) Karst) at Mg:Al molar ratios of < 0.2. In the hydroponic experiment (Rehmus et al. 2014a), Al induced toxicity symptoms occurred at treatments with Al concentrations in nutrient solution > 300 μM Al. From those Al treatments with 600 μM Al and higher, the Mg:Al and Ca:Al molar ratios in nutrient solution were < 0.2 and < 1, respectively. Thus, Al-driven Ca and Mg deficiency might be a reason for reduced biomass production and Ca:Al and Mg:Al molar ratios might be a possible indicator for Al stress to the investigated tree species. In the present study, only the Mg:Al molar ratios of SS15 and SS30 from MC 2.1 were < 0.2 but

distinctly higher in all other ecosystem solutions, suggesting problematic Mg:Al molar ratios only in deeper soil layers of MC 2.1 (Tab. 5.2). According to Cronan and Grigal (1995), the Ca:Al molar ratios of the two Melastomataceae species and the Lauraceae species which were < 12.5 (Tab. 5.2) would mean a 50 % and higher risk of Al stress. However, beneficial effects of Al on plant growth in hydroponic experiments with Al accumulating tree species, adapted to acid soils, have been reported (Osaki et al. 1997; Watanabe and Okada 2005). Only *O. bentamiana*, which is Al non-accumulating, might suffer immediate Al stress.

The Ca concentrations in tree leaves of the present study were all in the range required for optimal plant growth (1 – > 50 mg g^{-1}; Marschner 2012). From this comparison we conclude that there is no indication of a disturbed Ca nutrition. Rehmus et al. (2014b) reported a negative effect of increased Al concentrations on Mg uptake and translocation to the leaves of tropical forest trees. As Mg has a crucial function as the central ion in the chlorophyll molecule, impaired photosynthesis because of reduced Mg concentrations was assumed to be the reason for reduced biomass production. The Mg concentrations in the tree leaves from the study area (Tab. 5.1) are comparable to Mg concentrations in leaves of Brazilian Cerrado trees reported by Lilienfein et al. (2001) (0.71 - 2.1 mg g^{-1}) and in tree leaves of a tropical seasonal rain forest in southwest China by Shanmughavel et al. (2001) (3.2 - 5.4 mg g^{-1}). In leaves of 4 and 8 of the 17 analyzed tree species in our study Mg concentrations were below the limit values required for optimal growth of crop plants reported by Amberger (1996) (2 – 50 mg g^{-1}) and Marschner (2012) (1.5 – 3.5 mg g^{-1}), respectively. Thus, Mg deficiency might affect growth of some of the tree species. However, low Mg concentrations in trees might primary result from the Mg-poor bedrock and only secondary be result of the Mg-Al antagonism.

The mean base saturation in the soil A horizons was low and the mean Al saturation high (Tab. 5.3), which implies reduced supply with base cations for the benefit of Al ions. Because of the higher pH values in the valley bottom soils and lateral addition of leached base from above-lying soils, the mean base saturation was significantly higher in the valley bottom than the ridge top soils (Fig. 5.4). The ranges of base and Al saturation are complementary at respective pH values and show that the fraction of exchangeable Al can be high (up to 95 %). In

mineral soils, Al might have an indirect negative effect on plant nutrition via Al-P precipitation. However, soil P status can be improved by chelation of Al in organic complexes (Haynes and Mokolobate 2001). The resulting nutrient scarcity and high Al concentrations might be the reason for the low root length density in the mineral soils and high root length density in the organic layers (Soethe et al. 2006). Applying the critical base saturation of ≤ 15 % proposed by Cronan and Grigal (1995), out of the 23 analyzed soils in our study approximately 30 % might pose a potential risk of Al toxicity, particularly on the ridges, where 4 of the 7 soils with a BS < 15 % and the two soils with marginally higher BS (< 16 %) were located (Fig. 5.4), although Al concentrations are clearly below toxicity thresholds (Fig. 5.2).

5.6 Conclusions

We conclude that:

i) partly high Al fluxes, particularly in litterfall, indicate a high Al circulation through the ecosystem.

ii) Al concentrations in organic layer leachate are mainly driven by the acidification of the ecosystem and episodic base ion inputs during La Niña events. However, the Al concentrations in organic layer leachate are low.

iii) Al concentrations in tree leaves are high in accumulating species and Ca:Al molar ratios in organic layer leachate and leaves of most Al non-accumulating tree species are unproblematic. Yet, the fraction of exchangeable and potentially plant available Al in mineral soils is high, being a likely reason for a low root length density in the mineral soil. Hence, the ecosystem seems to be well adapted to the given Al concentrations and a wide distribution of Al accumulating tree species and high Al fluxes in the ecosystem do not necessarily imply a general Al phytotoxicity.

5.7 Acknowledgments

We thank Jürgen Homeier and Ingeborg Haug for their support in determining plant species, Thorsten Peters, Paul Emck, Rütger Rollenbeck, Jörg Bendix, and Michael Richter for providing climate data, Jaime Arturo García Ochoa for providing data on nutrient composition in leaves from the investigated study site, Daniel Kübler for providing maps for the localization of specific tree species in the forest, Alexander Hermann for the illustration of the Al fluxes, Nature and Culture International in Loja, EC, for providing the study area and the research station, Ecuadorian authorities for the research permit, and the German Research Foundation (DFG) for funding (FOR 816, Wi 1601/8-2).

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Appendix A.

The appendix is attached as a CD containing the following files:

Contents (contents.pdf)

Net plant growth (fresh weight) and root and shoot (stems and leaves) dry weight of *C. odorata*, *H. americanus*, and *T. chrysantha* seedlings (01_plant_weight.xlsx)

Root morphology parameters of *C. odorata*, *H. americanus*, and *T. chrysantha* seedlings (02_root_morphology.xlsx)

Healthy and diseased leaf area of *C. odorata*, *H. americanus*, and *T. chrysantha* seedlings (03_leaf_area.xlsx)

Element concentrations in leaves, stem, and roots of *C. odorata* seedlings (04_elements_Codorata.xlsx)

Element concentrations in leaves, stem, and roots of *H. americanus* seedlings (05_elements_Hamericanus.xlsx)

Element concentrations in leaves, stem, and roots of *T. chrysantha* seedlings (06_elements_Tchrysantha.xlsx)

Chemical composition of nutrient solution (07_nutrient_solution_chemistry.xlsx)

Chemical properties of organic layer leachate (LL) and mineral soil solutions in the 0.15 m (SS15) and 0.30 m (SS30) soil depth at the control and Ca amended plots of the NUMEX experiment as required for the modeling of

Al speciation and modeled concentrations of Al species and their proportion of total Al in solution (08_Al_speciation_NUMEX.xlsx)

Chemical properties of precipitation, throughfall, stemflow, and stream water from the study site MC 2 (09_aboveground_solutions_MC2.xlsx)

Chemical properties of organic layer leachate (LL) and soil solutions in the 0.15 m (SS15) and 0.30 m (SS30) soil depth from the study site MC 2 (10_soil_solutions_MC2.xlsx)

Element concentrations in litterfall from the study site MC 2 (11_litterfall_MC2.xlsx)

Annual Al fluxes and catchment budgets from April 1998 to March 2003 at the study site MC 2 (12_Al_fluxes_MC2.xlsx)

Element concentrations in tree leaves from the study area (13_elements_forest_trees.xlsx)

pH values ($CaCl_2$), base saturation, and Al saturation in soils from the sudy site MC 2 (14_base_Al_saturation_soils_MC2.xlsx)

Erklärung

gemäss Art. 28 Abs. 2 RSL 05

Name/Vorname:	Rehmus, Agnes
Matrikelnummer:	11-124-484
Studiengang:	Geographie
	Bachelor [ ] Master [ ] Dissertation [x]
Titel der Arbeit:	Aluminum toxicity in a tropical montane forest ecosystem in southern Ecuador
Leiter der Arbeit:	Prof. Dr. Wolfgang Wilcke

Ich erkläre hiermit, dass ich diese Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen benutzt habe. Alle Stellen, die wörtlich oder sinngemäss aus Quellen entnommen wurden, habe ich als solche gekennzeichnet. Mir ist bekannt, dass andernfalls der Senat gemäss Artikel 36 Absatz 1 Buchstabe r des Gesetzes vom 5. September 1996 über die Universität zum Entzug des auf Grund dieser Arbeit verliehenen Titels berechtigt ist.

Bern, den .....

Agnes Rehmus